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PREVALENCE OF DRUG-DRUG INTERACTIONS AND CLINICAL RELEVANCE FOR TREATMENT WITH ORAL ANTICOAGULANTS

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Cover illustration: Dabigatran, rifampicin, and diclofenac, three drugs with interactions that are studied in this thesis. Photo by Johan Holm 2020.

Prevalence of Drug-Drug Interactions and Clinical Relevance for Treatment with Oral Anticoagulants

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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ABSTRACT

The aim of this thesis was to describe prevalences and frequencies of potential DDIs in the Swedish outpatient population, and the clinical effects in treatment with oral anticoagulants.

Two cross-sectional studies were conducted to establish the prevalence and frequency of potential DDIs among Swedish outpatients. Study I included the whole outpatient population in Sweden, and study II the pediatric outpatient population. The prevalence of clinically relevant potential DDIs among patients with at least two drugs was 19 % and 1.4 % respectively. The proportions of clinically relevant DDIs that potentially lead to reduced treatment effect were 49 % of class D interactions (recommendation to avoid) and 54 % of class C interactions (may require e.g. dose adjustment) in the whole population. The corresponding proportions were 48 % and 32 % in the pediatric population. A limited number of drugs were involved in a large proportion of potential DDIs. Furthermore, many of the clinically relevant DDIs may lead to reduced treatment effect, an aspect of interactions that may be underestimated in clinical practice.

In study III, a cohort of warfarin patients was studied to analyze the longitudinal effects of initiation of amiodarone therapy on warfarin dose and INR. The mean weekly warfarin dose was 24.6 % lower after initiation of amiodarone (95 % CI, 23.5–25.6 %; $P < 0.001$). Mean weekly INR peaked the third week of concomitant treatment. The fraction of patients with an INR over 3 was 37.1 % at that point, compared to 11.7 % at baseline.

The increased risk of bleeding or thromboembolism, potentially associated with DDIs among patients treated with non-vitamin K antagonist oral anticoagulants (NOACs), was investigated with survival analyses in study IV. Atrial fibrillation outpatients were included in the cohort. Compared to patients not exposed to the interacting group of drugs, exposure to potential pharmacodynamic DDIs were associated with an increased risk of any severe bleed, for patients with apixaban HR (95 % CI) 1.47 (1.33-1.63), rivaroxaban 1.7 (1.49-1.92), and dabigatran 1.26 (1.05-1.52). In addition, exposure to CYP3A4 and/or P-gp inhibitors was associated with an increased risk of any severe bleed for patients treated with apixaban 1.23 (1.01-1.5). No significant effects could be established for patients exposed to inducers of CYP3A4 and/or P-gp.

In conclusion, one fifth of patients with at least two drugs in the whole Swedish outpatient population was exposed to potential DDIs. The identified drugs and potential clinical consequences need to be considered in clinical practice to avoid adverse events. Patients initiated on amiodarone during warfarin treatment had a mean dose reduction of 25 % and close monitoring during the first weeks is important to avoid supratherapeutic anticoagulant effect. Pharmacodynamic and pharmacokinetic DDIs with NOACs expose atrial fibrillation patients to increased risk of bleeding. These DDIs are important to consider from a risk benefit assessment perspective in patients treated with NOACs.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

- I. A limited number of prescribed drugs account for the great majority of drug-drug interactions.
Holm J, Eiermann B, Eliasson E, Mannheimer B.
European journal of clinical pharmacology. 2014;70(11):1375-83.
- II. Prevalence of potential drug-drug interactions in Swedish pediatric outpatients.
Holm J, Eiermann B, Kimland E, Mannheimer B.
PLoS One. 2019 Aug 5;14(8):e0220685. doi: 10.1371/journal.pone.0220685
- III. The effect of amiodarone on warfarin anticoagulation: a register-based nationwide cohort study involving the Swedish population.
Holm J, Lindh J D, Andersson M L, Mannheimer B.
Journal of thrombosis and haemostasis: JTH 2017;15(3):446-453.
- IV. Bleeding and thromboembolism due to drug-drug interactions with non-vitamin K antagonist oral anticoagulants - a Swedish, register-based cohort study in atrial fibrillation outpatients.
Holm J, Mannheimer B, Malmström R E, Eliasson E, Lindh J D.
European journal of clinical pharmacology. 2020 Oct 7. doi: 10.1007/s00228-020-03015-7.

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LIST OF ABBREVIATIONS

ACE inhibitors	angiotensin-converting enzyme inhibitors
ARB	angiotensin II receptor blockers
ASA	acetylsalicylic acid
ATC	anatomic therapeutic chemical
BCRP	breast cancer resistance protein
BSEP	bile salt export pump
COX	cyclo-oxygenase
CYP	cytochrome P450
DDD	defined daily dose
DDI	drug-drug interaction
DVT	deep venous thrombosis
EHRA	European Heart Rhythm Association
EMA	European Medicines Agency
FDA	Food and Drug Administration
HIV	human immunodeficiency virus
ICD	international classification of diseases
INR	international normalized ratio
LMWH	low molecular weight heparin
MATE	multidrug and toxin extrusion proteins

MRP	multidrug resistance related protein
NOAC	non-vitamin K antagonist oral anticoagulants
OAT	organic anion transporter
OATP	organic anion polypeptide
OCT	organic cation transporter
OTC	over the counter
PCI	percutaneous coronary intervention
PE	pulmonary embolism
P-gp	P-glycoprotein
SFINX	Swedish Finnish Interaction X-referencing
TTR	time in therapeutic range
UGT	uridine 5'-diphospho-glucuronosyltransferase

1 INTRODUCTION

The general theme of this thesis is drug-drug interactions (DDIs) and a specific focus is given to interactions with oral anticoagulants. Specifically, four studies have been conducted, two on exposure to potential DDIs and two on the clinical effects of DDIs in treatment with oral anticoagulants. Initially, before the study plan for the whole thesis was decided upon, we conducted the study I of exposure to potential DDIs in the whole Swedish population of outpatients. As we gradually formed the study plan for the thesis, we wanted to follow up on a number of research questions related to the general field of DDIs. In the study II we focus on the prevalence of DDIs in the pediatric subgroup of the population, that we expected would have a different mix of prescribed drugs compared to the whole population. Furthermore, we wanted to study the clinical outcome of DDIs. The DDIs of the many combinations of drugs that emerged from study I and II have a wide variety of potential clinical effects, increased risk of bleeding was one of the most frequent potential effects. Anticoagulants are widely used and DDIs can potentially result in dire consequences for patients. Increased risk of bleeding as well as reduced treatment effect and risk of thromboembolism are potential consequences of DDIs with anticoagulants. Consequentially, to study the clinical effects of DDIs we chose to focus on oral anticoagulants in study III and IV. In study III we focused on a single DDI between warfarin and amiodarone and the effect on dosing and INR. In contrast, study IV is focused on the whole group of non-vitamin K antagonist oral anticoagulants (NOACs) and the clinical outcome of interactions. Together, the four studies constitute different pharmacoepidemiologic approaches to study DDIs and the results give insights into the overall exposure to potentially interacting drugs in the outpatient population as well as specific clinical effects of DDIs with oral anticoagulants.

In the following text a background to the project will be given by a description of research on the epidemiology of clinical consequences of DDIs. Furthermore, mechanisms that lead to DDIs will be described as a background to how interactions occur. Databases and classification systems for DDIs, used in clinical practice and observational research will be presented. Evidence base and interaction databases as clinical tools and in relation to observational research will be discussed. The methodologies and results of the studies of this thesis will be presented and discussed in light of the results of others. Furthermore, the methodological advantages, challenges and limitations related to design of the studies in the thesis will be discussed.

From theoretical and methodological perspectives there are many ways to approach research on DDIs. Pharmacokinetic studies are necessary to understand the magnitude of influence on a drug's metabolism by an interaction. In addition, other types of studies contribute to establishing the clinical impact in a patient population. In the studies of this thesis we focus on the prevalence, frequencies, and clinical impact among patients, of drugs where there is

evidence suggesting DDIs. It is important to keep in mind that the basis for studies of clinical impact is the evidence of mechanistic impact. Consequentially, the overarching objective in this text and in the studies of the thesis is to understand to what extent interacting drugs are used in combination, despite knowledge of the interacting effect, and to study the clinical impact of known interactions with anticoagulants.

1.1 TERMINOLOGY

1.1.1 Drug-drug interaction

The terms “DDI” and “drug interaction” are not equivalent. DDIs result from the interaction between two drugs. The term “DDI” is restricted to interactions between drugs whereas the term “drug interaction” includes interaction effects on drugs by e.g. other drugs, foods, supplements, herbal remedies, or medical conditions and diseases. Consequentially, the term “drug interaction” is wider than the term “DDI” and the concept includes terms like “drug-food interaction” or “drug-disease interaction”. However, the term “drug interaction” is sometimes used pragmatically for brevity in a context where it can be understood to denote a DDI.

From a mechanistic perspective DDIs are generally classified as either pharmacokinetic or pharmacodynamic [1]. Pharmacokinetic interactions occur when one drug affects the systemic exposure level or tissue concentration of another drug in the body. These interactions occur when the absorption, distribution, metabolism, or excretion of a drug is affected [2]. Pharmacodynamic interactions by contrast, occur when the pharmacological effects of two drugs coincide, modulate or antagonize each other. This type of interactions can occur both at the target tissue and in other tissues by additive, synergistic or antagonizing effects, or adverse effects, of drugs.

1.1.2 Potential DDI and clinical effect

The terminology used when discussing DDIs in relation to clinical effects can be somewhat confusing. “DDI” is commonly used meaning interacting drug combinations that lead to actual clinical outcomes. The term “potential DDI” is used to denote a combination of drugs that have a mechanism for interaction but may or may not have consequences for the patient, i.e. the clinical effect is not what is focused on but rather the combination of drugs [3, 4]. Another term that is used is “drug-drug event”, i.e. the exposure to drugs that may interact with clinical significance. In contrast, a DDI is understood as a clinical event caused by an interacting combination of drugs. However, one could argue that if there is a pharmacological mechanism by which the drugs interact, an interaction occurs, whether the interaction leads to a clinically relevant event or not. A more intuitive terminology could denote combinations of

drugs with hypothetical interaction mechanisms that have not been established as “potential” or “theoretical”. Furthermore, DDIs with an established pharmacological mechanism could be denoted “DDI” and clinical effects of DDIs could be described as exactly that.

Regardless of this reflection on terminology, and adhering in a pragmatic way to convention, in the following text, the term “DDI” will be used as well as the term “drug interaction” to denote the concept “DDI”, and the reader will be trusted to understand the specific meaning of “drug interaction” from the context. Furthermore, “potential DDI” will be used in the conventional meaning of the term. In addition, the term “DDI” will also sometimes be used for brevity, denoting what are actually potential DDIs, again the context reveals the meaning. However, the term “DDI” will not be used to denote a clinical event resulting from an interaction without context. The term “drug-drug event” will not be used in this text.

1.1.3 Janusmed interactions and SFINX

In the studies of the thesis and in this text, the Janusmed interaction database is referred to by different names. The database was initially developed and maintained by Swedish and Finnish collaborators under the name SFINX (Swedish Finnish Interaction X-referencing). During the work with this thesis it was further developed into two distinct resources, Janusmed interactions and INXBASE [5, 6]. Therefore, both Janusmed interactions and SFINX are used to refer to the same database in the papers and this text, depending on the time of publication. In this text, the current name, Janusmed interactions, will be used. The INXBASE version of the database has not been used.

2 BACKGROUND

2.1 CLINICAL IMPACT OF DRUG-DRUG INTERACTIONS

The general clinical impact of DDIs has been investigated primarily in hospital settings. Clinically manifested DDIs had a pooled prevalence of 9.2 % (CI 95 % 4.0–19.7) in a recently conducted meta-analysis based on ten articles, of which eight were European. The meta-analysis included 6 540 patients and prevalence ranged between 1.2 % and 64.0 % in the individual studies. Patients from the emergency setting and internal medicine had lower prevalence than those in geriatric or intensive care. Studies performed using an electronic DDI database, confirming the DDI by tests or in medical records and analyzed by specialists, and containing data for calculation of prevalence were included. Notably, among 5 999 studies found in the search process only ten fulfilled these criteria, possibly indicating insufficient reporting or design in studies within the field. However, the approach may be too strict on the other hand and may not allow for different approaches that might be needed to identify clinical impact of DDIs within different settings. It is not unlikely that it is difficult to fully evaluate the clinical impact of multiple potential DDIs and handle the numerous and very different variables that must be quantified to correctly identify this outcome. Additionally, DDIs that lead to reduced effect of treatment may not be identified as clinically manifested DDIs in medical records [7]. A literature review, published in 2007, estimated the association of DDIs and visits at emergency departments, hospitalizations, and re-hospitalizations [8]. The review was based on studies published between 1990 and 2006. Overall, 23 studies were identified during the period that fit the inclusion criteria. Interestingly, large studies had low incidence rates and small studies had larger incidence rates, possibly a consequence of the problem of quantifying a heterogenous endpoint in large datasets mentioned above. 0.054 % of visits at emergency departments were due to DDIs, and in 0.57 % of hospital admissions and 0.12 % of re-hospitalizations DDIs were implicated. Percentages for hospitalization and rehospitalization among the elderly were higher than in the general population. Among the elderly, it was estimated that as much as 4.8 % of hospitalizations were due to DDIs. GI-bleeds, hypertension and hypotension, and cardiac rhythm disturbances were the most common reasons for visits at emergency departments. Furthermore, NSAIDs and cardiovascular drugs were commonly involved in DDIs causing these types of hospitalizations. In general, in both the meta-analysis and the review mentioned above, estimates from different studies of the clinical impact of DDIs vary considerably. Consequentially, the results need to be interpreted with caution.

Another review published in 2012, was based on the same studies to a large part as the one discussed above, but with a few additional studies [9]. In this review, studies are not summarized to give overall estimates of DDIs as a cause of hospital admissions. Rather the perspective of clinical impact is discussed based on the type of data. Data from primary healthcare and other outpatient settings is rare and again the review is mainly based on hospital admissions. However, the authors conclude that, based on the limited data, clinical

impact of DDIs seems to be lower in outpatient than inpatient settings. DDI incidence in studies based on visits to emergency departments range between 0 % and 0.17 %, whereas incidence based on hospital admissions range between 0.12 % and 4.3 % for all age groups and 0.67 % and 6.2 % in the elderly. One study based on patients younger than 18 years report an incidence of 0.009 %. Incidence based on rehospitalization data range between 0 % and 7.6 % in the studies reviewed. Fewer studies report on incidence of DDIs during hospital stay. Incidence estimates between 6.9 % and 14.3 % are reported from studies conducted in intensive care, medical or surgical wards. Notably, the highest estimate came from a study in medical wards. One study was conducted in an intensive care unit on patients aged > 18 years and reported an incidence of 5.3 %. In general, the incidences reported in these studies are based on different study periods and consequentially should be compared with caution.

Studies conducted in pharmacovigilance databases are few [9]. Varying percentages, between 1 and 35.5 %, of adverse reactions were connected to DDIs depending on the population and drugs studied. Since data from adverse drug reaction registers are based on spontaneous reports, the results cannot be directly understood as a measure of how common DDI-related adverse reactions are in the population.

As mentioned above, studies estimating the impact of DDIs in medical records data cannot be expected to find all clinically relevant DDIs. DDIs that cause lower grade symptoms may go unnoticed but can still be clinically relevant and cause harm to the patient. This may lead to noncompliance to treatment, that the patient may not report the symptoms, or that the symptoms may not be understood as connected to a DDI by the patient or by healthcare. Furthermore, loss of treatment effect, that may not be understood as connected to a DDI by healthcare, can be another reason for misclassification in these kinds of studies [7]. Additionally, there may be a risk of misclassification in large studies of DDIs based on medical records or databases, due to healthcare practitioners not documenting a DDI as the reason for a clinical condition though it might in fact be the true reason for what occurred. Consequentially, there are several reasons why the kind of studies discussed above may in fact underestimate the true clinical impact of DDIs. However, as mentioned in the meta-analysis from 2020, it is worth noting that the high prevalence of potential DDIs that are sometimes reported do not correspond to equally high prevalence of clinically manifested DDIs [10]. This is not unexpected given the nature of mechanisms behind DDIs which is further discussed below.

Estimating clinical impact of DDIs by studies on healthcare consumption or DDIs documented in medical records is probably a crude way of understanding a problem that is multifactorial and lead to a wide variety of clinical events for which it is difficult to find

relevant and detailed endpoints. It is therefore not unlikely that the problem is underestimated. However, the expected conclusion that not all clinically relevant potential DDIs lead to adverse events is important and must be considered when weighing the risks and benefits of treatment.

2.2 HEALTHCARE COSTS AND DRUG-DRUG INTERACTIONS

It is reasonable to assume that clinically relevant DDIs leading to adverse events increase the costs of treating patients. However, the peer-reviewed literature on the economic consequences of DDIs is limited. Few studies can be found that focus on healthcare cost in relation to DDIs in general. A Brazilian retrospective cross-sectional analysis from a public hospital published in 2009 found independent associations between length of stay, number of drugs, higher comorbidity index, hospitalization costs, and exposure to potential DDIs [11]. Comorbidity and polypharmacy are potential confounders that may influence the analysis of healthcare costs. However, based on the independent association found for costs in this analysis the authors argue that exposure to potential DDIs may lead to adverse events that increase length of stay and costs. In a retrospective, matched cohort study from 2011, based on patients enrolled in the Mississippi Medicaid program, patients exposed to potential DDIs were matched to controls based on use of the drug affected by the interaction, demographic, and comorbidity variables [12]. ACE inhibitors, ARBs, beta blockers, clonidine, and warfarin were the drugs involved in DDIs with the greatest economic effects on hospitalizations, emergency room visits, average per patient hospital payments, and average per patient emergency room payments. Furthermore, a nested case-control study of patients with atrial fibrillation and long-term warfarin treatment from 2012 found that patients who were prescribed interacting drugs had higher treatment costs related to hemorrhages than patients without interacting drugs [13]. Increased costs associated with potential DDIs have also been shown in observational studies among users of opioids [14, 15], benzodiazepines [16], methotrexate or cyclosporine [17], antiretroviral drugs [18], and antipsychotics [19].

There are obvious challenges in determining the costs presumably caused by the DDIs. From a methodological perspective a few confounding factors may be difficult to handle. As described above comorbidity and polypharmacy may be related to increased costs and an increased risk of exposure to interacting drugs. In the studies cited above different measures have been taken to control for these factors. However, there is an obvious risk of residual confounding due to the difficulty of characterizing patients completely in data from large registers. Furthermore, few of the studies cited above connect exposure to a potential DDI with an adverse event that can be reasonably assumed to follow from the interaction and cause the extra cost. This is a difficult chain of events to capture accurately in large database studies. Nevertheless, evidence of the clinical impact of interactions as well as high

prevalence of potential DDIs inevitably leads to an assumption that costs are increased, and this is a field of study in need of more evidence.

2.3 MECHANISMS FOR DRUG-DRUG INTERACTIONS

2.3.1 Pharmacokinetic drug-drug interactions

Pharmacokinetic drug-drug interactions occur when one drug affects the exposure to another drug. Principally a pharmacokinetic drug interaction can occur at any level of absorption, distribution, metabolism, or excretion of the drugs [20]. The evidence for clinical relevance varies for the different types of pharmacokinetic DDIs [2]. Consequentially, pharmacokinetic drug interactions that are most often discussed and perceived as relevant in the clinical setting can be found primarily among the DDIs involving drug absorption, Cytochrome P450 (CYP) metabolism, and P-gp transport.

2.3.1.1 Absorption

DDIs that affect drug absorption are the results of changes in either the rate or extent of absorption. When the rate of absorption of one drug is influenced by another drug, the clinical effect of the drug may be delayed. Additionally, the effect can be reduced if the total amount of absorbed drug is reduced due to gastrointestinal transit time in relation to the change in absorption rate. A reduction of absorption of more than 20 % is generally considered clinically significant [21]. Other mechanisms by which absorption may be influenced include changes in gastrointestinal pH, chelation of drugs, and effects on intestinal CYP enzymes or transport proteins. Drugs that are dependent on gastric pH for dissolution and for which absorption is limited based on the solubility of the drug, have a reduced absorption when an antacid or a PPI is administered concomitantly [22]. When metallic cations and organic molecules are administered concomitantly chelation can cause reduced absorption. Coadministration of tetracyclines and antacids constitute an example of this interaction [23]. The effect may however be counteracted by separated administration in time of the two drugs [22].

2.3.1.2 Plasma proteins

Highly protein bound drugs may theoretically displace other highly protein bound drugs, and this could cause a change in drug distribution. However, few clinically relevant DDIs have been found that result from an altered protein binding. Elimination of displaced drugs depend on the extraction ratio of the eliminating organ and most drugs that are highly protein bound have low extraction ratios [24]. From a therapeutic drug monitoring perspective however, DDIs resulting in an altered free fraction of a drug may be relevant for interpretation.

2.3.1.3 *Metabolism*

Inhibition or induction of enzymes for drug metabolism are major mechanisms for pharmacokinetic DDIs. The effect on the enzyme results in decreased or increased metabolism of substrate drugs. Drugs are metabolized by two types of reactions, phase I and phase II reactions. Phase I reactions, e.g. oxidation, reduction, and hydrolysis result in compounds that can be further metabolized. Phase II reactions often result in inactive compounds by biotransformation and combination of the drug with another substance e.g. by glucuronidation, sulfation, methylation, acetylation, or glycine conjugation [2].

The rate of drug metabolism is decreased by enzyme inhibition, this is a more common mechanism for DDIs than enzyme induction [20]. Inhibition leads to accumulation and a higher exposure to the drug in the body which may cause adverse events and toxicity [2, 20]. If the drug is a prodrug, with less activity than the metabolite produced, inhibition can lead to loss of pharmacological effect. The effect of enzyme inhibition can develop within 2-3 days of exposure [20]. It can be reversible or irreversible. If the inhibition is reversible, enzyme activity is regained as the inhibiting substance is cleared from the body, i.e. the enzyme activity depends on the elimination half-life of the inhibiting substance. Inhibition can be competitive or noncompetitive, depending on whether the inhibitor and the substrate competes for the active site on the enzyme or not. Irreversible inhibition results from permanent inactivation of the enzyme by the inhibitor. The time to regaining enzyme activity then depends on the synthesis of new enzyme, which is a slower process [2].

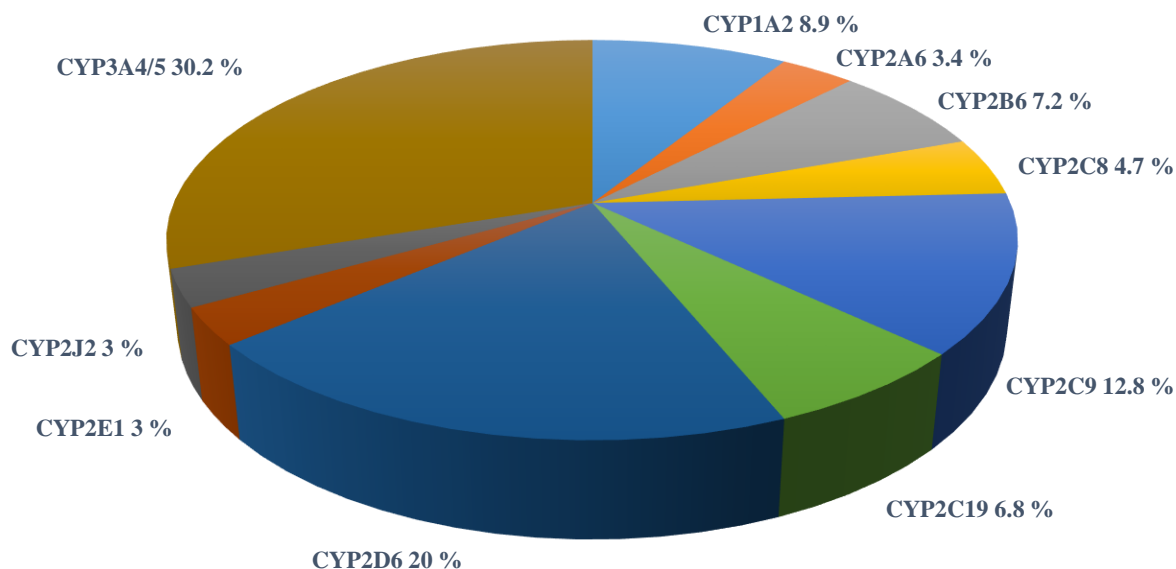
Enzyme induction results from increased synthesis of the induced enzyme. Induction of metabolic enzymes result in an increase in intestinal or hepatic clearance of the substrate drug. The result is a lower exposure and potentially reduced effect, depending on the therapeutic range of the drug. Induction may also cause accumulation of a toxic metabolite and result in toxicity [2]. Enzyme induction takes days or weeks to develop fully since it depends on increased synthesis of the enzyme, which is a slower process compared to the mechanism for inhibition [20].

The clinical impact of inhibition or induction of a metabolizing enzyme depend on both the effect of the inhibitor or inducer on the enzyme and on the extent to which the substrate is sensitive to the effect on the enzyme and metabolized by the enzyme. An inhibitor or inducer can have strong, moderate or weak effect on the metabolizing enzyme [20]. Index substrates are used to measure the effect of a drug as an inhibitor or inducer. Strong inhibitors increase the AUC of index substrates of a metabolic pathway ≥ 5 -fold, moderate < 5 to ≥ 2 -fold, and

weak <2 to ≥ 1.25 -fold. Strong inducers decrease the AUC of index substrates of a metabolic pathway by $\geq 80\%$, moderate by $\geq 50\%$ to $<80\%$, and weak by $\geq 20\%$ to $<50\%$ [3, 25]. In addition substrate sensitivity to inhibition or induction varies between different drugs [20]. Furthermore, if there are different metabolic pathways for the substrate drug and the enzyme affected by the inhibitor/inducer is involved in only one of these, the impact on exposure depends on the extent of the drug that is metabolized through the enzyme [2].

The CYP metabolic enzymes are central in phase I metabolism of drugs and interactions involving these enzymes account for many clinically relevant DDIs. CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2C8, and CYP2C19, roughly in order of importance based on the number of interactions, are the most commonly CYP enzymes involved in drug metabolism (figure 1). A few drugs are also known to be metabolized by e.g. CYP2B6 and CYP2E1. About 90 % of the metabolism of the commonly used drugs are metabolized by these enzymes [20]. CYP3A4 is highly important for drug metabolism and is involved in the metabolism of more than 50 % of drugs used clinically [26]. Examples of substrates can be found in various drug groups e.g. antineoplastics, azoles, benzodiazepines, calcium-channel blockers, corticosteroids, opioids, protease inhibitors and statins [20]. It is also involved in the metabolism of NOACs, studied in the fourth paper of this thesis [27]. Strong inhibitors include ketoconazole and HIV-protease inhibitors, strong inducers include carbamazepine, phenytoin and rifampicin. CYP2D6-substrates include e.g. some antipsychotic drugs, betablockers, opioids, and tricyclic antidepressants. Examples of strong inhibitors are quinidine, cinacalcet, fluoxetine, and paroxetine, whereas carbamazepine and rifampicin exemplify strong inducers. Substrates of CYP2C9, can be found in drug groups such as NSAIDs, statins and sulfonylureas. The interaction between warfarin and amiodarone is studied in the third paper of this thesis. *S*-warfarin is a substrate of CYP2C9, and amiodarone is a moderate inhibitor. Examples of other inhibitors are fluconazole and miconazole whereas e.g. rifampicin and enzalutamide are examples of inducers. CYP2C19 is involved in the metabolism of many drugs to some extent. Substrate drugs with extensive metabolism by CYP2C19 are e.g. omeprazole, citalopram and diazepam. Fluconazole, fluoxetine and fluvoxamine exemplify strong inhibitors and rifampicin and apalutamide strong inducers. Substrates of CYP1A2 include e.g. duloxetine, caffeine, and melatonin, whereas fluvoxamine is a strong and ciprofloxacin a moderate inhibitor. Phenytoin and rifampicin induce CYP1A2 to a moderate extent [20].

Figure 1. Fraction of clinically used drugs metabolized by CYP isoforms. Data published in 2013, note that this is a general overview and may not correspond in detail to the distribution of drugs used today. Only the major metabolic pathway for each drug was counted. Adapted from Zanger et al, published under Creative Commons license [28].



Among enzymes involved in phase II reactions less is known about clinically relevant DDIs [20]. UGTs, responsible for glucuronidation, are inhibited or induced by a number of drugs [20, 29-31]. Examples of inducers are rifampicin and ritonavir, and inhibitors include e.g. valproic acid and probenecid [29-31]. Though there are examples of clinically relevant interactions through UGTs, in many cases interactions through UGTs do not cause clinically relevant effects [29].

2.3.1.4 Transport proteins

Interactions may also occur by induction or inhibition of transport proteins. P-gp, OATPs, OATs, MATEs, OCTs, MRPs, BSEP, and BCRP are all involved in drug transport and may be influenced by drugs and other xenobiotics, and potentially cause interactions that affect the disposition of the substrate drugs. The relevance for DDIs for P-gp is most well-known among these transporters whereas for other transporters the clinical relevance needs more exploration [2].

P-gp is a transport protein involved in clinically relevant DDIs. P-gp can be found in organs that have barrier or elimination functions and have a role in first-pass drug metabolism of orally administered drugs [32]. It is located in the luminal epithelia of the gut, the bile facing

canaliculi of the liver, the proximal tubules of the kidney, and the blood-brain barrier [33]. P-gp is an important efflux pump and promotes lowering of systemic levels of its substrates. There is an overlap between drugs that interact with P-gp and CYP3A4. Both enzymes can be found in the same organs and tissues and have complementary functions in reducing drug exposure. However, the effect of a drug on the respective enzyme are not always equally potent [32]. Some of the NOACs, studied in the fourth paper of this thesis, exemplify drugs that to varying extent are susceptible to interactions through both P-gp and CYP3A4 [27]. Though the overlap between P-gp and CYP3A4 substrates, inhibitors, and inducers is extensive, there are examples of drugs that are P-gp substrates but not CYP3A4 substrates, e.g. digoxin, dabigatran and talinolol [32, 34]. Numerous substrates, inhibitors, or inducers of P-gp can be found e.g. among anticancer and immunomodulatory drugs, cardiovascular drugs and anticoagulants, drugs for infectious diseases, CNS drugs, and drugs for gastrointestinal or endocrine diseases [32].

The OATPs and OATs are primarily influx transporters, in contrast to e.g. P-gp or MRP, and consequentially inhibition may lead to decreased exposure of substrate drugs whereas induction may lead to increased concentrations [2]. OATPs are located e.g. in the gastrointestinal tract, kidney, liver, and the blood-brain barrier. In the intestine, inhibition of OATPs may theoretically cause reduced absorption of a substrate drug. A number of common fruit juices are inhibitors of intestinal OATPs [35]. OATP substrates include fexofenadine and digoxin, but the clinical relevance of OATP on DDIs with these drugs is unclear. Examples of drugs interacting with OATPs are statins, cyclosporin and ketoconazole [2]. OATs are involved in the renal secretion of drugs but can also be found in hepatocytes and in the intestine [34]. Leflunomide, probenecid, pravastatin, cimetidine, cephalosporins, teriflunomide, and some NSAIDs are examples of OAT inhibitors [2, 34]. A number of drugs, e.g. benzylpenicillin and adefovir are believed to be substrates of OATs [34]. However, in many cases effects are not believed to be clinically significant though there may be exceptions to this e.g. for probenecid and cephalosporins [2]. OCT and MATE are involved in the active secretion of drugs in the kidney and bile. Cimetidine and trimethoprim are examples of OCT and MATE inhibitors and there is some evidence that clinically relevant interactions may involve effects on OCT and MATE [34]. MRP can be found in the liver, intestine, and kidney where it is involved in hepatobiliary and renal elimination of drugs. It has been hypothesized to have a role in the DDI between vincristine and piperacillin but the clinical relevance is not clear [34, 36]. The role of BSEP as a drug transporter is not well defined and its relevance to DDIs is not clear. However, it is inhibited by e.g. ciclosporin, glibenclamide, and bosentan, it is involved in the secretion of bile salts, and inhibition may increase the risk of cholestasis [34, 37]. BCRP is located in the gastrointestinal tract, liver, and kidneys [34]. It has been implicated in DDIs leading to an increased exposure to rosuvastatin [34, 38].

2.3.1.5 Renal excretion

Changes in urinary pH and renal blood flow by drugs may cause pharmacokinetic changes to the exposure to other drugs that are primarily renally excreted. In addition, as described above, DDIs may be caused by effects on the function of transport proteins in the kidney. Exposure to acidic drugs, e.g. phenobarbital, aspirin and salicylates, can be decreased by antacids or bicarbonate that change the urinary pH. However, before renal excretion most drugs are metabolized to inactive compounds and changes in urinary pH does not influence elimination [2, 37]. Drugs affecting renal function may inhibit the excretion of other drugs. An example of this type of DDI is inhibition of tubular secretion by NSAIDs that may lead to increased methotrexate exposure and toxicity, though the clinical impact of the interaction has been discussed [39, 40]. Furthermore, it has been suggested that a change in renal blood flow may lead to reduced excretion of some drugs by inhibition of prostaglandin synthesis by e.g. NSAIDs [37].

2.3.2 Pharmacodynamic drug-drug interactions

Pharmacodynamic DDIs, in contrast to pharmacokinetic DDIs do not result from a change in drug exposure due to the interacting drug. Instead, the interaction occurs due to the effects of the interacting drugs at the target sites i.e. the pharmacological effects of a drug is influenced by the effect another drug [2, 41]. These type of interactions can result in additive, synergistic or antagonistic effects [2]. Additive effects occur when the overall effect of the combination of drugs is the sum of the effects of the individual drugs. Synergistic effects are greater than the sum of the pharmacological effects of the combined drugs. Antagonistic effects occur when the pharmacological effects of the interacting drugs counteract. Pharmacodynamic additive, synergistic, or antagonistic effects can influence the desired effect of drugs as well as adverse effects and toxicity [41]. An example of a synergistic beneficial pharmacodynamic interaction is the combination of leucovorin and fluorouracil in cancer therapy. The combination results in synergistic cytotoxicity by an increased inhibition of thymidylate synthase [42]. Other examples are the synergistic effect of combining antibiotics to treat an infection or the combination of NSAIDs with SSRIs that increase the risk for gastrointestinal hemorrhages [2, 43]. An example of relevance to the fourth paper of this thesis is the combination of a NOAC with aspirin or clopidogrel, that may result in both wanted and unwanted additive effects on hemostasis [44].

2.3.3 Mechanisms for drug-drug interactions with oral anticoagulants

Drugs that affect the metabolism of oral anticoagulants through pharmacokinetic interactions can result in an increased risk of bleeding or a reduction of the anticoagulant effect and thus an increased risk of thromboembolism. Furthermore, pharmacodynamic interactions with drugs that affect hemostasis may increase the risk of bleeding. Warfarin is well known for its

interaction potential, which pose clinical problems [45]. In recent years, the NOACs have become increasingly used as alternatives to warfarin for oral anticoagulant treatment [46–49].

2.3.3.1 Pharmacokinetic drug-drug interactions with oral anticoagulants

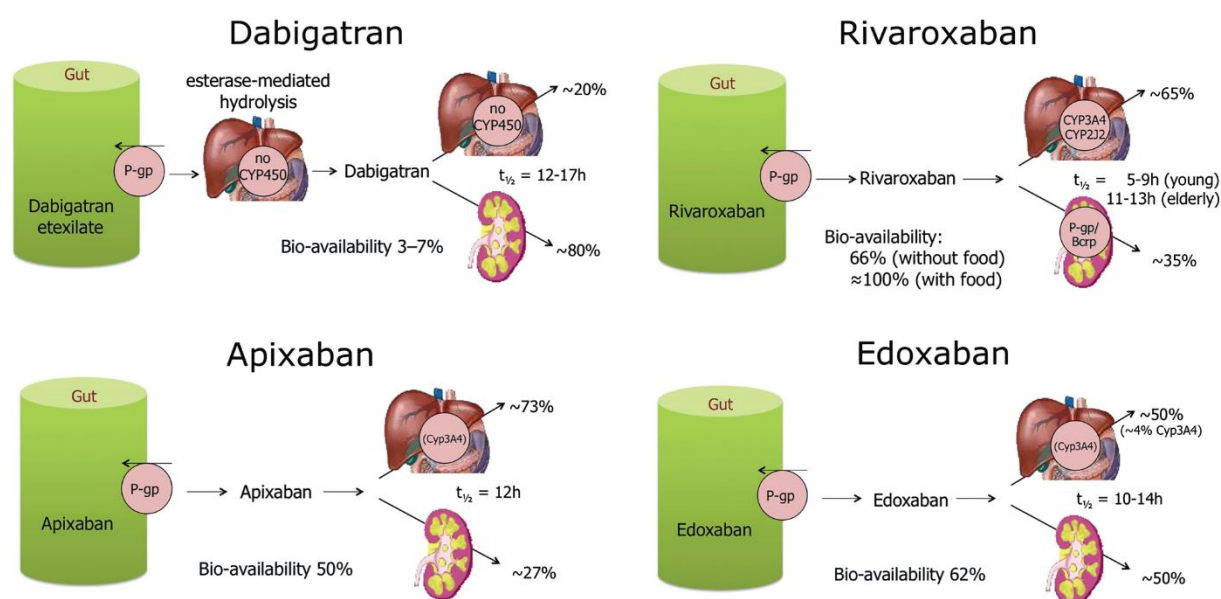
Warfarin has many clinically relevant DDIs that may result in both increased bleeding risk and reduced anticoagulant effect [45]. Warfarin is a racemic mixture of two isomers, *S*- and *R*-warfarin. *S*-warfarin is more potent than *R*-warfarin. Both isoforms are metabolized by CYP enzymes. The *S*-enantiomer is primarily metabolized by CYP2C9 and the *R*-enantiomer by CYP1A2 and CYP3A4 [50]. Warfarin is eliminated as inactive metabolites in urine and stool [26]. Pharmacokinetic interactions between warfarin and drugs that inhibit or induce these enzymes can be found among commonly used drug groups, e.g. anti-infectives, immune modulators, cardiovascular and neurologic drugs [45, 50]. Furthermore, there are several pharmacokinetic interactions between warfarin and food or dietary supplements [50]. The interaction between warfarin and amiodarone, studied in paper three, is a pharmacokinetic DDI that leads to higher warfarin concentration and an increased risk of bleeding.

Amiodarone inhibits both CYP2C9 and CYP1A2. Consequently, it inhibits warfarin in a non-stereoselective manner, though the effect on *S*-warfarin is more important for the effect due to its higher pharmacological potency [51]. Furthermore, the interaction with amiodarone exemplify how the interacting effect of a drug with prolonged elimination can remain after discontinuation of the drug. Amiodarone has a half-life of weeks to months and the inhibiting effect on CYP2C9 and CYP1A2 can remain long after discontinuation of treatment [26, 52].

The NOACs currently marketed in Sweden are apixaban, rivaroxaban, edoxaban, and dabigatran [53]. Figure 2 provides an overview of the metabolism of different NOACs. Apixaban is primarily metabolized by CYP3A4/5. To some extent CYP1A2, 2J2, 2C8, 2C9, and 2C19 are also involved in its metabolism [54]. The larger part of the dose, 50 % and 27 % respectively, is eliminated unchanged through intestinal/biliary and renal excretion. Since metabolic clearance is not the primary way of elimination many CYP-based DDIs are less likely to be clinically relevant. However, concomitant treatment with strong inhibitors or inducers of CYP3A4 may lead to clinically relevant interactions. Apixaban is also a substrate to the P-gp and BCRP transporter proteins [54, 55]. Pharmacokinetic drug interactions are expected primarily based on P-gp and CYP3A4 inhibition or induction and strong inhibitors or inducers of both CYP3A4 and P-gp can lead to significant changes in exposure [50, 55]. Apixaban exposure is increased by renal dysfunction, in patients with severe renal dysfunction AUC increased by 44 %, but the risk of bleeding is less compared to other NOACs for patients with eGFR <50 ml/min [56]. Like apixaban, rivaroxaban is a CYP3A4/5-substrate. Additionally, CYP2J2 has a smaller role in its metabolism [54, 55]. Furthermore, it is a P-gp and BCRP substrate and like apixaban treatment with inhibitors and inducers of CYP3A4 and P-gp may affect its metabolism and result in pharmacokinetic

interactions [50, 54, 55]. Due to multiple clearance pathways, interactions with rivaroxaban based on CYP3A4 or P-gp can be expected to have clinical relevance primarily for strong inhibitors or inducers [54]. After oral administration, about 65 % of the drug is metabolized. [55, 57]. Edoxaban is a P-gp substrate and, like the other NOACs, it is susceptible to pharmacokinetic DDIs via drugs that affect P-gp [58]. Elimination of edoxaban is primarily in unchanged form, about 70 %, and metabolism is mainly through carboxylesterase-1, while CYP3A4/5 account for a smaller part of its clearance [55, 58]. Consequentially, clinically relevant DDIs based on CYP inhibition or induction are normally not expected [55, 58]. About 50 % of the systemically available dose of edoxaban is eliminated renally [59]. Dabigatran is not metabolized by CYP enzymes. However, the prodrug dabigatran etexilate is a substrate of the transport protein P-gp and consequently pharmacokinetic interactions can occur with drugs that inhibit or induce P-gp [50, 55]. Dabigatran etexilate is metabolized to dabigatran, the active compound, by esterase-catalyzed hydrolysis [55]. About 80 % of dabigatran is eliminated in urine and therefore renal function is an important determinant of dabigatran exposure [60].

Figure 2. Absorption and metabolism of the different NOACs. Interaction possibilities include the level of absorption, first transformation, metabolism, and excretion. Reproduced with permission, Heidbuchel et al [61].



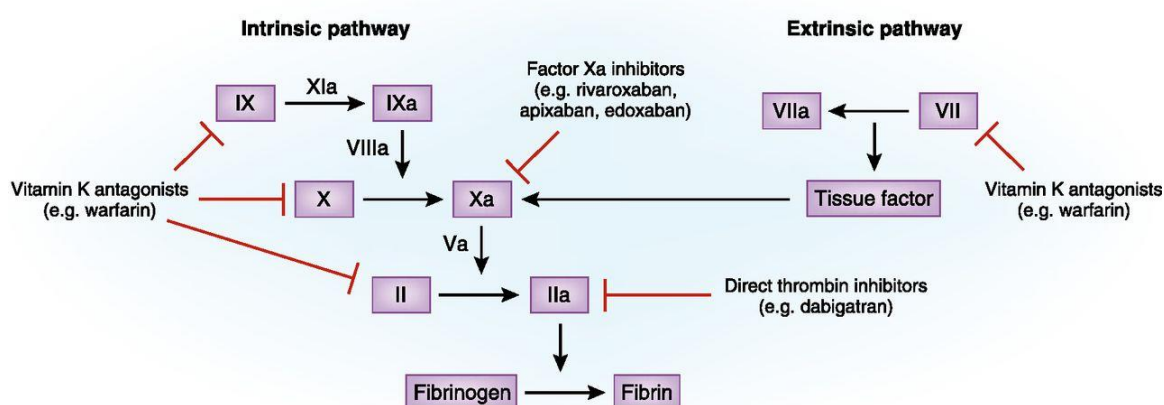
2.3.3.2 Pharmacodynamic drug-drug interactions with oral anticoagulants

The pharmacological effect of warfarin is achieved by a lowering of the amount of active vitamin K, needed for the activation of coagulation factors II, VII, IX, and X. These coagulation factors need carboxylation to be activated and vitamin K is needed for this process. Warfarin blocks vitamin K epoxide reductase (VKOR) and thereby interferes with

vitamin K conversion which leads to production of lower amounts of active coagulation factors [62].

Apixaban, rivaroxaban, and edoxaban are all factor Xa inhibitors. Factor Xa is a coagulation factor in the coagulation cascade and the rate limiting step for thrombin generation. The pharmacological effect of rivaroxaban and apixaban is due to the unchanged form of the drugs, whereas edoxaban has pharmacologically active metabolites. However, since relative concentration of the metabolites is low the effect is mainly attributable to unchanged edoxaban. Dabigatran in contrast, is a direct thrombin inhibitor. Dabigatran etexilate, the prodrug of dabigatran, does not contribute to the pharmacological effect [55]. Conjugation with glucuronic acid of about 20 % of plasma dabigatran produces pharmacologically active metabolites [60]. The sites of action for different oral anticoagulants in the coagulation cascade is given in figure 3.

Figure 3. The coagulation cascade and sites of action for warfarin, dabigatran, apixaban, rivaroxaban, and edoxaban. Reproduced with permission, Jain et al [63].



Drugs that have anticoagulant effects interact pharmacodynamically with warfarin and the NOACs by additive or synergistic effects on bleeding. Below, the pharmacodynamic effects of antiplatelet drugs, heparins, NSAIDs and SSRIs are given as examples but generally all drugs that increase bleeding risk can interact pharmacodynamically with oral anticoagulants, though the clinical effect on bleeding risk differ between different drug groups. In addition, it should be noted that some of these drugs also have pharmacokinetic interactions with warfarin or NOACs.

Antiplatelet therapy with ASA or P2Y₁₂ inhibitors, e.g. clopidogrel or ticagrelor, leads to an increased risk of bleeding [44, 64, 65]. However, shorter periods of combination therapy with warfarin or NOACs after coronary interventions with PCI are used for secondary prevention, despite the increased bleeding risk due to the risk-benefit balance with recurring acute coronary syndrome. Doses and suitable drug combinations are discussed though [66-68].

Heparin and LMWHs enhance the effect of antithrombin, which is an endogenous anticoagulant that inhibits the coagulation cascade by interaction with factor Xa and thrombin (factor II) [26, 69]. Combination with other anticoagulants such as warfarin or NOACs lead to increased risk of bleeding and switching between different types of anticoagulants requires separation of doses to mitigate the risk of a pharmacodynamic interaction effect [70].

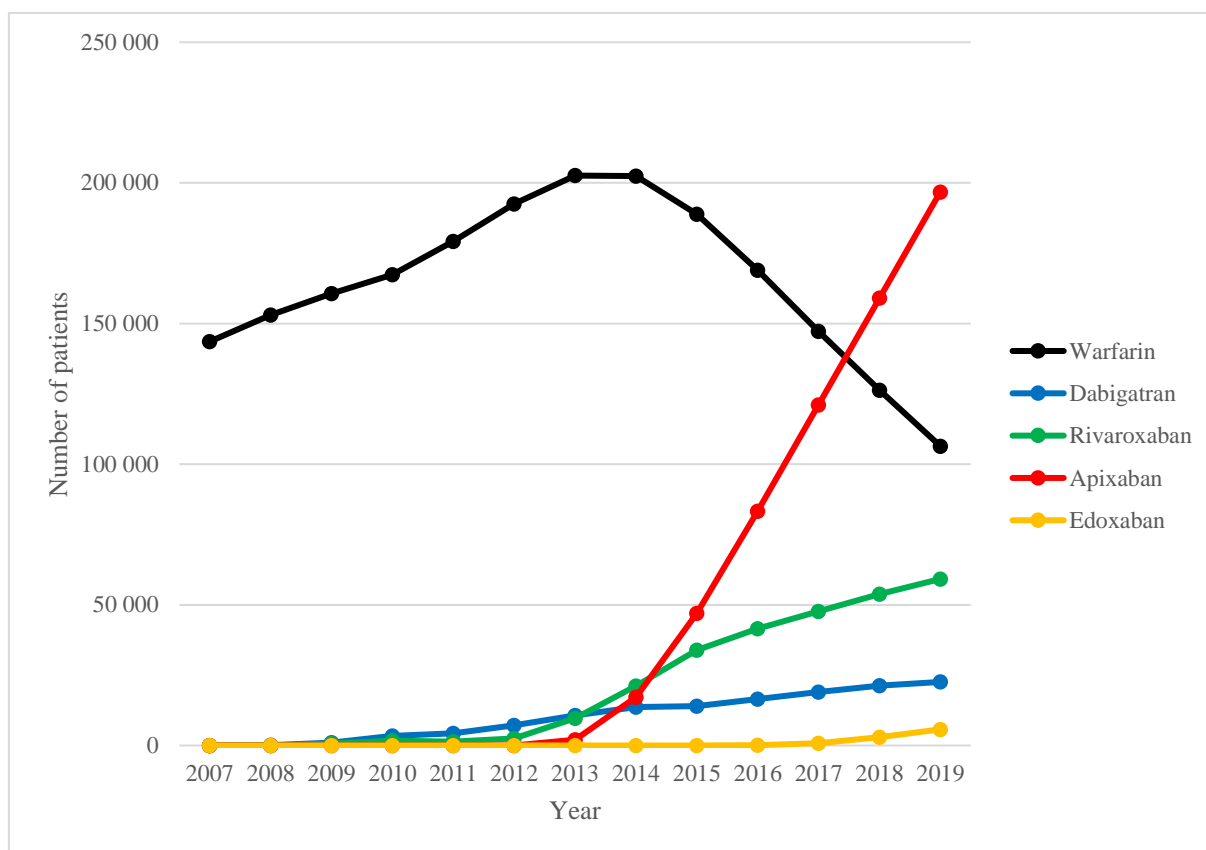
NSAIDs have an anticoagulant effect similar to that of ASA. The inhibition of platelet COX lead to a reduced production of thromboxane 2 which has a role in activation and aggregation of platelets [69, 71]. Unlike ASA, NSAID inhibition of COX is reversible and the effect on bleeding is shorter [69]. Combination of NSAIDs and anticoagulants consequentially increase the risk of bleeding [72].

Serotonin have a role in platelet activation and aggregation [69, 71, 73]. Serotonin is taken up from plasma, stored in platelets and released upon activation [71]. SSRIs inhibit the uptake of serotonin into platelets which results in an antiplatelet effect. Furthermore, serotonin mediates increased gastric acidity that may contribute to ulcers [73]. Consequentially, concomitant treatment with anticoagulants and SSRIs may lead to increased risk of bleeding [74].

2.4 DISPENSATION OF ORAL ANTICOAGULANTS OVER THE PAST YEARS

In this thesis, a specific focus is given to oral anticoagulants regarding the clinical impact of potential DDIs. Studies have been conducted on warfarin as well as the newer NOACs. Since the introduction of NOACs, an increasing number of patients are treated with drugs from this group, rather than with warfarin [75]. However, though apixaban is used to treat more patients in later years and the number of patients treated with NOACs are increasing, warfarin is still widely used in Sweden (figure 4).

Figure 4. The number of patients with dispensed oral anticoagulants in Sweden per year, 2007 to 2019 (data from the Prescribed Drug Register) [75].



2.5 EVIDENCE BASE AND INTERACTION DATABASES AS CLINICAL TOOLS AND IN RELATION TO OBSERVATIONAL RESEARCH

From the preceding sections it is evident that not all interactions between drugs that occur mechanistically lead to changes in pharmacodynamics or pharmacokinetics that translate into clinically relevant effects. Furthermore, clinical impact can vary depending on therapeutic interval of the drug, and patient-specific characteristics such as genetic polymorphisms of the relevant drug metabolizing enzymes or diseases that the patient may have [76, 77]. This is not to say that only potential DDIs that frequently lead to clinical effects are the only types worth considering from a clinical perspective. In the interaction database Janusmed interactions, class B interactions, i.e. interactions where the clinical outcome of the interaction is uncertain and/or may vary, exemplify this category of interactions [78]. Furthermore, variations in clinical impact occur within all categories of DDIs. Though clinicians may not always act on a potential DDI, the consideration and weighing of risks and benefits with treatment need to rely on good evidence of what is known about the potential effects [79].

The European and North American medicines agencies, EMA and FDA, provide guidelines for the investigation of drug interactions related to development of drugs [3, 4, 25]. These

guidelines, and relevant regulations for industry, on how to investigate potential DDIs with developed drugs determine much of the information generated on DDIs. In addition, academic researchers add to the evidence available as the drug is introduced to the market. As described above, the level of effect of an inducer or inhibitor on the substrate drug determines whether a pharmacokinetic interaction is considered significant or not. For both pharmacokinetic and pharmacodynamic interactions, evidence of clinical effects connected with concomitant treatment is central. However, the level of evidence for a specific DDI may vary between results from studies based on drugs with similar theoretical potential for interactions, or single case reports, to well-structured controlled studies on the relevant drug combination in a relevant study population [78].

Several DDI databases or clinical decision support systems are available globally to guide prescribers on DDIs. Many of these resources have been integrated into electronic medical records systems and provide alerts on DDIs to clinicians while prescribing. Integration into medical records systems have been shown to lead to reduced prescribing of potential DDIs [80]. However, it has also led to a discussion on the problem of over-alerts that may lead to alert fatigue and potentially decreased use of the resource [10, 81]. Selective alerts and minimized workflow interruptions may improve user acceptance [81]. It is important to note though, given the differential clinical impact of DDIs for different patients and in different settings described above, that overrides of alerts do not necessarily imply that the warning have not been evaluated by the prescriber. From the perspective of research on prevalence of DDIs, interaction databases are frequently used to identify relevant potential DDIs in studies. Examples of resources frequently used in observational studies are Hansten and Horn, Lexi-Comp, Micromedex, Stockley's Drug Interactions, and Epocrates [10, 82, 83]. Janusmed interactions is a Swedish interaction database and decision support system. It is integrated into several medical records systems in the country, and is used in observational research [80, 84].

The results of observational studies are influenced to a considerable extent by the database used to identify and classify DDIs. An overview of the classification of DDIs in three resources frequently used in observational research is given as examples in tables 1-3. Though these classification systems have considerable similarities, the differences have the potential to translate into different results in observational studies, depending on the database used. To exemplify, consider the interaction between dabigatran and phenytoin, an inducer of P-gp mediated dabigatran excretion. In Janusmed interactions this is classified as a C2-interaction, not the highest level of clinical impact since dabigatran plasma concentration can be monitored and dose adjustment considered [5]. In Stockley's Drug interactions it is classified as a severe, theoretical interaction, and monitoring is recommended [85]. This is essentially concurrent evaluations, but because of the differences in classification systems the clinical impact category of the classification in Janusmed interactions is not the highest,

whereas in Stockley's Drug Interactions due to the potential severity the interaction is classified as a severe interaction, the highest level of clinical impact. In Micromedex, the interaction is classified as major with good documentation [86]. Comparing to the other two sources, this again is a concurrent evaluation that result in a different classification. The clinical impact category is the highest, differing from Janusmed interactions but not from Stockley's Drug Interactions, whereas the documentation class is the second highest. In Stockley's Drug Interactions it is the lowest whereas in Janusmed interactions it is in the middle of the range. These differences exemplify how the same information fit different classifications differently. Depending on the interaction database chosen, an observational study will include this DDI in different classes of interactions and results may consequentially differ on an aggregated level, not because of differences in the clinical context but because of this methodological aspect. This is an important aspect of the methodological background to observational studies to be aware of when interpreting results from research in the field.

Table 1. Overview of the DDI classification system for Janusmed interactions. Any category of clinical impact can be combined with any category of documentation for the evaluation of a specific DDI [78].

Janusmed interactions			
Clinical impact		Documentation	
D	Clinically relevant interaction. The combination is best avoided.	4	Data derived from controlled studies in relevant patient populations.
C	Clinically relevant interaction that can be handled e.g. by dose adjustments.	3	Data derived from studies among healthy volunteers and/or pilot studies among patients.
B	Clinical outcome of the interaction is uncertain and/or may vary.	2	Data derived from well-documented case reports.
A	Minor interaction of no clinical relevance.	1	Data derived from incomplete case reports and/or in vitro studies.
		0	Data derived from extrapolation on the basis of studies with similar drugs.

Table 2. Overview of the DDI classification system for Micromedex. Any category of clinical impact can be combined with any category of documentation for the evaluation of a specific DDI [86].

Micromedex			
Clinical impact		Documentation	
Contraindicated	The drugs are contraindicated for concurrent use.	Excellent	Controlled studies have clearly established the existence of the interaction.
Major	The interaction may be life-threatening and/or require medical intervention to minimize or prevent serious adverse effects.	Good	Documentation strongly suggests the interaction exists, but well-controlled studies are lacking.
Moderate	The interaction may result in exacerbation of the patient's condition and/or require an alteration in therapy.	Fair	Available documentation is poor, but pharmacologic considerations lead clinicians to suspect the interaction exists; or, documentation is good for a pharmacologically similar drug.
Minor	The interaction would have limited clinical effects. Manifestations may include an increase in the frequency or severity of the side effects but generally would not require a major alteration in therapy.	Unknown	Unknown.
Unknown	Unknown.		

Table 3. Overview of the DDI classification system for Stockley's Drug Interactions. Any category of clinical impact can be combined with any category of documentation and action for the evaluation of a specific DDI [85].

Stockley's Interaction Alerts

Clinical impact		Documentation		Action
Severe	Interactions that could totally incapacitate a patient or result in either a permanent detrimental effect or a life-threatening event.	Extensive studies	Based on numerous small or medium size studies or several large studies. The information is usually supported by case reports.	Avoid
Moderate	Interactions that could result in an effect that may either cause considerable distress or partially incapacitate a patient. These interactions are unlikely to be life-threatening or result in long-term effects.	Formal study	Based on formal study. This may be one small or medium size study, or several small studies. The studies may or may not be supported by case reports.	Adjust
Nothing expected	Interactions that are unlikely to result in an effect, or for drugs pairs where no interaction occurs.	Case reports	Based either on a single case report or a limited number of case reports. No trials appear to have been conducted.	Monitor
		Theoretical	Based on a theoretical interaction or lack of interaction. This information may have been derived either from in vitro studies involving the drug in question or based on the way other members of the same group act.	Information
				No action

3 RATIONALE

Interactions between drugs constitute a complex and to a large extent preventable problem related to drug treatment. As described above, DDIs may occur at all levels of absorption, distribution, metabolism, or excretion of drugs. Clinical impact varies depending on mechanistic factors. Additionally, the clinical impact of DDIs vary, depending on inter- and intraindividual factors. Consequently, DDIs constitute a complicating factor to drug treatment and evidence on which interactions patients are exposed to as well as the clinical impact of DDIs is a field in constant need of research as new drugs are developed. The general rationale for focusing on DDIs in the thesis was to contribute to the understanding of drug use in the context of the complex problems that DDIs entail. The field is broad and multifaceted, and the first two studies focus on general prescribing of interacting drugs. From a study design perspective, an overarching approach with DDIs related to all drugs prescribed would be difficult to maintain in studies of the clinical consequences of DDIs, since different DDIs have very different outcomes suitable for study in different sources and with various approaches. For studies III and IV the perspective was therefore narrowed to oral anticoagulants. The rationale for this focus was the widespread use of anticoagulants, the high potential risks connected to interactions with anticoagulants, and the emergence of new anticoagulants on the market at the time when the thesis was planned [27, 75].

When study I was planned no large inventory of exposure in the whole Swedish outpatient population to clinically relevant DDIs had been made since 1999 [87]. A few studies had been conducted in the elderly population in the past years [88, 89]. A recent study of the whole population had shown increasing polypharmacy (5 or more dispensed prescriptions) by 8.2 % and increase of excessive polypharmacy (10 or more dispensed prescriptions) of 15.7 % during the period between 2005 and 2008 in data from the Prescribed Drug Register [90]. Consequentially, we perceived that a cross-sectional study, describing clinically relevant potential DDIs that were prevalent among Swedish outpatient, would add new information on the use patterns of interacting drugs and highlight potential risks that patients are exposed to. Additionally, it would present an opportunity to describe in greater detail the drugs most often involved in clinically relevant potential DDIs.

Study II was conducted on the pediatric subset of the population in study I. Previously, studies had been conducted in specific pediatric patient groups as well as in general in- and outpatient settings, but few studies could be found that described potential DDIs in a general pediatric population including primary healthcare [91-101]. Differences in drug use between pediatric and adult patients as well as the relative scarcity of previous studies in general pediatric outpatient settings motivated an analysis focused on the pediatric subset of patients to further elucidate the use of potentially interacting drugs in Swedish outpatients.

Amiodarone, a class III antiarrhythmic, is an inhibitor of warfarin metabolism that potentially increase warfarin exposure with concomitant treatment [51]. Since both drugs are used to treat patients with atrial fibrillation, they are frequently used concomitantly and the combination has been associated with an increased risk of bleeding [102, 103]. At the time of planning the study, only a few small longitudinal studies on the effect on ongoing warfarin treatment by the introduction of amiodarone were available [52, 104-106]. Consequentially, a study based on a large number of patients would add precision to the understanding of the effect of amiodarone on warfarin anticoagulation.

Dabigatran was introduced to the Swedish market in 2008, as the first drug in the NOAC group. Since then the use of these drugs has increased markedly in Sweden and abroad, and NOACs have become first-line treatment for stroke prevention in atrial fibrillation [27, 75, 107, 108]. Polypharmacy is frequent among these patients and consequentially exposure to DDIs may add to the complexity of treatment [109-111]. There are few observational studies on the overall clinical impact of potential DDIs with NOAC and pharmacokinetic studies are the primary source for guidelines and recommendations [111-113]. A retrospective study, based on all patients with atrial fibrillation in Sweden for whom NOAC treatment had been initiated since the introduction in 2008, could therefore be expected to add information on the clinical impact of DDIs with NOACs in healthcare.

4 OBJECTIVES

4.1 GENERAL OBJECTIVES

The overall objective of the thesis was to study DDIs from perspectives of exposure in the population as well as clinical impact. Prevalence and frequencies of potential drug interactions and involved drugs were to be described in the whole population, including all ages, and in the pediatric population specifically. Furthermore, to study clinical impact the perspective was narrowed to focus on oral anticoagulants in studies on effects of DDIs on dosing, monitoring, and adverse events.

4.2 SPECIFIC OBJECTIVES

4.2.1 Study I

To study the frequency of clinically relevant potential DDIs in prescription drugs dispensed to Swedish outpatients. Furthermore, to describe common potential clinical effects of drug-drug interactions. In addition, to identify specific drugs frequently involved in DDIs.

4.2.2 Study II

To describe frequency of clinically relevant potential DDIs in Swedish pediatric outpatients based on dispensed prescription drugs. Furthermore, to describe the prevalence of clinically relevant DDIs in patients exposed to at least two drugs. In addition, to describe drugs frequently involved in DDIs, potential clinical effects of DDIs, and potential differences between different age-groups in exposure to DDIs.

4.2.3 Study III

To analyze the potential difference in weekly warfarin dose before and after initiation of amiodarone treatment. Furthermore, to descriptively analyze the weekly change in warfarin dose and INR after initiation of warfarin treatment.

4.2.4 Study IV

To analyze the difference in risk of adverse events for patients treated with NOACs with or without drugs that have clinically relevant potential interactions with each respective NOAC. Specifically, to analyze the difference in risk of severe bleeding events between patients treated with each NOAC, with or without potential pharmacodynamic interactions or pharmacokinetic interactions with CYP3A4 and/or P-gp inhibitors. In addition, to analyze the

difference in risk of thromboembolic events between patients treated with each NOAC, with or without CYP3A4 and/or P-gp inducers.

5 METHODS

5.1 DESIGN OVERVIEW

The studies in this thesis are all observational and retrospective. Some aspects of methodology are similar, e.g. the use of the Prescribed Drug Register and the database Janusmed Interactions, while other aspects differ between studies. Paper I and II describe cross-sectional studies of co-prescribing of potentially interacting drugs. Paper III describe a cohort study of longitudinal effects of the interaction between warfarin and amiodarone on INR and warfarin dose. Paper IV describe a cohort study of the risk of bleeding and thromboembolism associated with DDIs with NOACs. These different research approaches require different methods and entail different methodological problems. In the following section a brief overview of study design is given for each study. Furthermore, in table 4 the core aspects of study design are outlined. The overview is followed by a more elaborate general description of important aspects of methods applied in the different studies.

5.1.1 Study I

The first study was a retrospective, cross-sectional study based on data from the Prescribed Drug Register [114]. Data were retrieved from the register on all drugs dispensed between January 1 to April 30, 2010. The register contains information on all prescription drugs dispensed at pharmacies in Sweden. Data on drug dispensation were linked to information on interacting drugs from Janusmed Interactions, to identify concomitant use of interacting drugs in the population [78]. The resulting drug combinations were aggregated according to the potential effect of each interaction and sorted into drug groups based on the ATC classification system [115]. Clinically relevant interactions that can be handled, e.g. by dose adjustments (class C), and clinically relevant interactions that should be avoided (class D) according to Janusmed Interactions were analyzed separately. The association of age, sex and number of drugs to exposure to D-interactions was analyzed by logistic regression.

5.1.2 Study II

The second study was a retrospective, cross-sectional study based on the pediatric subgroup of the population in study I. Prescribed drugs dispensed to individuals aged 0 to 17 years (inclusive) were included. Methods for linking, classification, and aggregation of interacting drug combinations were the same as in study I. In contrast to study I, the association of age, sex and number of drugs to exposure to C- or D-interactions was analyzed with logistic regression.

5.1.3 Study III

The third study was a retrospective cohort study. Data on warfarin dosing and INR were retrieved from Auricula and Journalia [116, 117], two warfarin monitoring registers. Data on amiodarone dispensation and other concomitant drugs were retrieved from the Prescribed Drug Register [114]. Information from the two registers was linked by personal identity numbers, unique to each individual in Sweden. Patients dispensed other interacting drugs with clinically relevant effect on warfarin pharmacokinetics according to Janusmed Interactions were excluded [78]. Weekly mean INR and weekly dose in relation to baseline dose were calculated for the first 30 weeks after initiation of amiodarone. The mean daily warfarin dose at baseline, during four weeks before initiation of amiodarone, was compared to the four-week period 120 to 147 days after the index date, based on the descriptive analysis of when the interaction effect had developed. Mean change in log-transformed dose was compared to no change in a two-sided dependent t-test. The association of age and sex with the impact of amiodarone on warfarin dose was analyzed with linear regression. The fraction of patients with INR above 3 and 4, respectively, and the fraction of patients with a decreased warfarin dose by > 10 %, > 25 % or > 50 % were calculated.

5.1.4 Study IV

The fourth study was a retrospective cohort study based on data on dispensed prescribed drugs from the Prescribed Drug Register, and outcome data from the Patient Register and the Cause of Death Register [114]. Information from the three registers was linked by personal identity numbers. Data from these registers were retrieved for all patients dispensed a NOAC from the introduction of the drug group in 2008 until 2017. Drug dispensation data were retrieved from 2007 until 2017, data on diagnoses from the Patient Register from 1998 until 2017, and data on diagnoses and deaths from the Cause of Death Register from 2008 until 2017. Drug treatment periods were identified by linking of consecutive dispensations. Patients with other indications for NOAC treatment than atrial fibrillation were excluded. Patients with a newly initiated NOAC treatment were included, with a washout period for vitamin K receptor antagonists and previous NOAC treatment. Interacting drugs were identified based on clinical relevance according to Janusmed Interactions and European Heart Rhythm Association (EHRA) guidelines, and grouped according to the mechanism for interaction with each NOAC [27, 78]. The association between bleeding or thromboembolism and concomitant exposure to individual NOACs and interacting drug groups was analyzed with Cox-regression survival analysis. Exposure to NOACs and drug groups that interact with NOACs was compared to exposure to NOACs without the interacting drug groups during the first six months of treatment. The primary outcomes were any severe bleed for interactions with a potential effect on bleeding and ischemic stroke/transient ischemic attack/stroke unspecified for interactions that potentially result in reduced NOAC exposure. Secondary outcomes were gastrointestinal bleeding, intracranial bleeding, ischemic stroke, and venous thromboembolism.

Table 4. Design overview of studies in the thesis.

Study	I	II	III	IV
Design	Cross-sectional	Cross-sectional	Cohort	Cohort
Data sources	The Prescribed Drug Register	The Prescribed Drug Register	Auricula, Journalia, the Prescribed Drug Register	The Prescribed Drug Register, the Patient Register, the Cause of Death Register
Inclusion criteria	Exposure to C or D interaction	Exposure to C or D interaction	Warfarin treatment with new initiation of amiodarone	Treatment with NOAC
Exclusion criteria	-	-	Concomitant treatment with drugs that interact with warfarin, absence of repeated dispensation of amiodarone, absence of information on INR the month before index date	DVT, PE, knee or hip replacement within 60 days of index date, mitral insufficiency or mechanical valve
Study population	Swedish outpatients with clinically relevant DDIs (class C or D)	Swedish pediatric outpatients with clinically relevant DDIs (class C or D)	Warfarin-treated patients initiated on amiodaron	Atrial fibrillation outpatients with NOAC treatment
Number of patients	601 774	3 243	754	244 597
Study period	January 1 to April 30, 2010	January 1 to April 30, 2010	2005 to 2014	2008 to 2017
Primary outcomes	Frequencies of C and D interactions	Frequencies of C and D interactions	Change in warfarin dose	Any severe bleed and ischemic stroke/stroke unspecified/TIA
Statistical methods	Logistic regression	Logistic regression	T-test, linear regression	Cox-regression

5.2 DATA SOURCES

The retrospective studies in this thesis were conducted based on data from large national registers. In study I and II no linking of personal data was needed as all personal data came from the Prescribed Drug Register. In contrast, study III and IV required linking of personal data from different registers. In Sweden, all individuals have a unique personal identity number. All registers with personal data used in the thesis contain the personal identity number of each individual and consequently data from the registers were linked using these identifiers.

The Prescribed Drug Register is a register of all prescription drugs dispensed at pharmacies or drugs taken from drug storage rooms in nursing homes in Sweden [118]. It is maintained by the Swedish National Board of Health and Welfare. All pharmacies in the country must report dispensations that are included in the register, and more than 100 million posts are recorded each year. The register contains information on the patient, e.g. sex, age, and place of residence, the drug, e.g. ATC-code, drug name, strength, and size of the package, the prescription, e.g. amount, date of the prescription, date of the dispensation, cost, i.e. for the patient and healthcare, and the prescriber, e.g. where the prescription was made, and the profession and specialty of the prescriber [115, 118]. The register has a good coverage of outpatient drug dispensation in Sweden. However, OTC drugs or drugs used in hospital inpatient care are not included in the register [118, 119]. Drug dispensation data from The Prescribed Drug Register were used in all studies of the thesis. In studies I, II and IV all data on drugs came from the register. In study III data on warfarin dose and INR were taken from Auricula and Journalia, described below, whereas data on all other drugs were retrieved from the Prescribed Drug Register.

Auricula and Journalia are registers used for warfarin monitoring [116, 117]. Auricula is a national quality register for patients with atrial fibrillation and anticoagulant therapy [116]. It is a combination of quality register and a dosing and control system for anticoagulant treatment. Journalia AK is a monitoring register for anticoagulant treatment, other products not relevant for the thesis are also available from Journalia [117]. At the time of study III, over 300 Swedish anticoagulation clinics were using these systems. The registers contain information on the daily warfarin dose. This is an important difference to the Prescribed Drug Register that contain information on the whole dispensed prescription and consequentially cannot be used to study changes in daily dosing. Furthermore, the registers contain INR-values, sex, and age of the patients included [116-118]. Data on warfarin dosing and INR-values from Auricula and Journalia were utilized in study III.

The Patient Register contain information on all inpatient healthcare visits in Sweden since 1987. Furthermore, all outpatient care in specialized healthcare is covered since 2001 [120]. The register does not contain information on primary healthcare or patients treated only by other healthcare personnel than physicians [120]. Diagnoses are recorded according to the ICD system [121]. It also includes procedure codes, and dates for diagnoses and procedure codes [120]. The Patient Register has been validated for a number of diagnoses. Validity is generally good but varies between diagnoses [122-126]. A general review found positive predictive values of about 85-95 % for different diagnoses in the register [124]. Data on diagnoses and procedure codes from the Patient Register were used in study IV.

The Cause of Death Register include all deaths that occur in Sweden [127]. Data on the time of death and the cause of death are available in the register. Causes of death are recorded as ICD-diagnoses [121, 127]. Furthermore, diagnoses for conditions contributing to the cause of death are recorded [127]. Data from the register on deaths and diagnoses contributing to death were used in study IV.

5.3 STUDY POPULATIONS, INCLUSION AND EXCLUSION

The purpose of studies I and II was to describe co-prescription of potentially interacting drugs at the time of the studies in the whole Swedish outpatient population and in the pediatric outpatient population respectively. As described above, the Prescribed Drug register includes all dispensed prescription drugs dispensed at Swedish pharmacies [118]. Consequentially, a cross section of all data on dispensed prescribed drugs for the whole population was to be included from the register. The study population was therefore determined by the way the time frame for the cross section was defined. Due to the Swedish reimbursement model, patients on long-term drug treatment are dispensed drugs every three to four months. A representative cross section of concomitant drug treatment consequentially could not be defined as all dispensed drugs at a certain day. Instead a time frame would have to be used that captured consecutive dispensations of different drugs that constituted what prescribers and patients intended as concomitant treatment. We therefore defined the time frame for the cross section as four months between January 1 and April 30, 2010. All patients with dispensed prescription drugs recorded in the Prescribed Drug Register during this time period were included in the study population. The definition of the time frame in relation to concomitant treatment is further described under definitions of exposure below. For the purpose of defining the study population, the consequence of a shorter time period would have been a smaller population while a longer period would have resulted in a larger population. All definitions need to be considered in relation to potential misclassification and in relation to what constituted a cross section. This is further discussed in the methods discussion.

In study III, the need for detailed warfarin dosing and INR data required use of information more detailed than that available in the Prescribed Drug Register and the Patient Register. The study base was patients with stable warfarin treatment with a new episode of concomitant amiodarone treatment, and with no other drugs interacting with warfarin. The warfarin monitoring registers Auricula and Journalia, were widely used at the time, as described above, and consequentially these registers were suitable sources of data. Furthermore, in contrast to studies I and II inclusion needed to be stricter to identify a study population for which data were available that allowed detailed analysis of the endpoints. Patients ≥ 18 years of age with warfarin treatment recorded in Auricula or Journalia were considered for inclusion if they had concomitant amiodarone in dispensation data from the Prescribed Drug Register. Index date was the date for initiation of amiodarone treatment. A dispensation of warfarin in the Prescribed Drug Register, 28-140 days before the first dispensation of amiodarone, was used as a criterion to confirm treatment. Due to the pharmacokinetics of the interaction between amiodarone and warfarin (see pharmacokinetics section above) a washout period of 12 months for previous amiodarone dispensation was applied. Furthermore, to include patients with stable warfarin treatment, previous dispensation during the 4 to 20 weeks preceding index date was an inclusion criterion. In addition, second and third dispensations of amiodarone within 60 to 120 days, and 150 to 210 days were required, for inclusion only of patients with ongoing amiodarone treatment after index date. Exclusion criteria were concomitant treatment with any drug, four months before until 69 days after index date, that according to the Janusmed Interaction database may lead to a change in INR or warfarin AUC of more than 10 % [5]. Furthermore, patients were excluded if data on warfarin dosing or INR during the 28 days preceding index date were missing since this was needed for the analysis. In cases where more than one episode of concomitant treatment fulfilled the above criteria, the first episode was included for each patient. Consequentially, the study population consisted of all patients, identified in Auricula or Journalia, on stable warfarin treatment who had amiodarone treatment initiated that continued for the duration of the study, who were not exposed to other drugs with potentially clinically relevant interaction effects on warfarin, and for whom data were available for analysis.

In study IV, the intention was to include the whole population of atrial fibrillation patients treated with NOACs in outpatient care. In contrast to studies I and II, data from more than one register was needed to identify the population. Furthermore, each NOAC was analyzed by itself and patients could have more than one episode of NOAC treatment during the study period, which complicates the definition of the study population. Information on drug treatment was available in the Prescribed Drug Register until 2018 whereas complete data in the Patient Register and Cause of Death Register were available until 2017 only. Consequentially, the study period for which data on both exposure variables and outcome variables were available was 2008 to 2017. Inclusion criterion was treatment with any NOAC recorded in the Prescribed Drug Register during the study period. From this selection of

patients, the first treatment period with apixaban, rivaroxaban, dabigatran, or edoxaban for each patient that did not fulfill the exclusion criteria was selected for the analysis of each NOAC. Exclusion criteria were presence of mitral insufficiency or mechanical valve, and any indication for NOAC treatment apart from atrial fibrillation within 60 days of index date, i.e. hip or knee replacement, DVT or PE. Furthermore, a washout period of 90 days was applied for treatment with a vitamin K receptor antagonist or previous NOAC treatment. A patient could therefore contribute with only one treatment period to the analysis of each NOAC, but possibly with different treatment periods to the analysis of different NOACs. Consequentially, the study population was defined as any patient treated with a NOAC between 2008 and 2017 who had at least one treatment episode with NOAC for which exclusion criteria were not fulfilled. The selection of inclusion and exclusion criteria is further discussed in the methods discussion.

5.4 CATEGORIES AND STRUCTURE OF INTERACTIONS

In studies I, II and IV multiple DDIs are studied in large populations. As described above, the clinical impact of DDIs vary, and a level of clinical relevance for inclusion in the studies needed to be defined. Furthermore, due to the large number of drugs and DDIs studied, relevant groups of interactions needed to be defined to allow analysis and comprehensive description of the results.

The Janusmed Interaction database was used in all studies to identify drugs combination for which there was evidence of an interaction [78]. The classification system in the database is described above in the background section. In study I, II and IV the intention was to include DDIs with evidence of, or reasonable pharmacological foundation for, clinically relevant interactions. Consequentially, class C and D interactions were included in the studies whereas class A and B interactions were not. The database also classifies the evidence behind each potential DDI, this classification was not used in the studies as a basis for inclusion of interactions. In study IV, in contrast to studies I and II, EHRA guidelines were considered in addition to the information on interacting drugs in Janusmed interactions [5, 27]. Interactions with clinically relevant effect listed in any of those sources, either as contraindicated or where dose adjustments or other measures are recommended to mitigate the interaction effect, were included.

In studies I and II, interactions were grouped according to the potential clinical effect for descriptive purposes. Two main groups were defined, i.e. adverse effect-type interactions and reduced effect-type interactions i.e. loss of effect from one or both drugs involved. Furthermore, subgroups were defined within those main groups based on the potential clinical consequence of the interaction, e.g. hypotension or reduced anticoagulant effect.

Notably, this grouping did not take pharmacological mechanism for the interaction into account, pharmacokinetic and pharmacodynamic interactions occur in the same consequence-based groups. In addition, it should be noted that the interacting drug pairs, i.e. the interactions were classified. Furthermore, interacting substances were grouped within the consequence-based groups based on ATC-code levels and drug substance groups [128]. In contrast, in study IV substances that interact with NOACs were grouped based on the pharmacological mechanism of the potential interaction for analytical purposes. Three groups were identified, drugs that lead to pharmacodynamic interactions, drugs that interact by inhibition of CYP3A4 and/or P-gp, and drugs that interact by induction of CYP3A4 and/or P-gp. For pharmacodynamic interactions, a substance included in this group for any NOAC based on Janusmed or EHRA guidelines was included for all NOACs [5, 27]. In contrast, only drugs where evidence indicated a clinically relevant interaction for the specific NOAC were included in the respective group for drugs that interact by inhibition or induction of CYP3A4 and/or P-gp. The rationale for this was that any pharmacodynamic interaction with impact on one NOAC can be assumed to have similar impact on all, whereas pharmacokinetic interactions can have very different impact on different NOACs due to different pharmacokinetic properties, as described in the section on mechanisms for drug-drug interactions above.

5.5 DEFINITIONS OF EXPOSURE

In all studies of this thesis retrospective register data is used to capture exposure to interacting drug combinations. The actual exposure of patients to interacting drugs is estimated by data from the registers that document dispensation of prescribed drugs. In studies I and II, data on dispensed prescription drugs were evaluated based on whether they interact. Drugs were identified based on the substance included in the product. Additionally, drugs were evaluated based on the form of administration to avoid inclusion of drug forms that do not expose patients to clinically relevant potential DDIs. However, apart from the dimension of exposure to single drugs, concomitant exposure needs to be estimated based on batch dispensations at different time points. Patients collect a number of doses at one time point, possibly of different drugs, and may collect a number of doses at later time points that are used concomitantly. Consequently, a time period needs to be defined during which all drugs that are dispensed are assumed to be concomitantly used to study what was intended as, and may be, concomitant use. In studies I, II, and III, a dispensation is assumed to represent roughly four months of drug use. The rationale behind this assumption, as mentioned above, is that due to the Swedish reimbursement model long term drug treatment is commonly dispensed every three to four months. In study I and II concomitant drug treatment with interacting drugs was defined as dispensations two drugs that interact according to Janusmed interactions and were dispensed within the four-month study period. The assumption was that all drugs that a patient use during this time period will be dispensed at some time during the period.

Duplicate dispensations of the same drug during the study period was eliminated to avoid duplicated interaction pairs for the same patient.

By the same rationale, in study III new dispensations of amiodarone within 60–120 and 150–210 days from the first were defined as ongoing amiodarone treatment during the period. The first amiodarone dispensation of a new treatment period preceded by 12 months without amiodarone dispensation constituted the index date. Treatment with warfarin was defined differently in study III, based on warfarin dosing schedules from Auricula or Journalia, since data on daily changes in dose were needed in the analyses [116, 117]. Concomitant treatment was defined as overlapping treatment periods with warfarin and amiodarone.

In study IV, a development of the concept of ongoing treatment based on consecutive dispensations was applied. In contrast to studies I and II, study IV was a survival analysis of the first six months of concomitant treatment with interacting drugs after initiation of co-treatment. Drug treatment was identified in the Prescribed Drug Register based on ATC-codes [115]. Only codes denoting forms of administration relevant to drug interactions with NOACs were included. Furthermore, ATC-codes sometimes change over time and consequentially historical ATC-codes relevant for the study period were also included [129]. Drug treatment episodes were estimated by linked consecutive dispensations considering the number of days that were covered by each prescription. However, the prescribed dose was not available from the data. Instead, the DDDs of each dispensation were assumed to be the time frame for treatment. A second dispensation was regarded as linked to the previous if it occurred within $2.5 \times$ the number of DDDs of the previous dispensation unless that number exceeded 100 days. The rationale for this way of estimating drug treatment periods from dispensations was that it would reduce the risk of interpreting shorter treatment periods that were not connected as continuous treatment compared to assuming that all dispensations represent three or four months of treatment. However, a DDD is a crude measure of the actual daily dose, in many cases the patient is prescribed a different dose. By multiplying DDD with 2.5, a dispensation of a prescription for half the DDD would still be connected to the next. However, by setting the maximum days between treatments to 100 days, prescription of a large dose compared to DDD would not be falsely connected to a second dose much later. Again, the limit of 100 days was chosen based on the presumed three to four months between dispensations described above. Exposure to a DDI was defined as the overlap of treatment periods of NOACs and interacting drugs based on the inclusion of DDIs described above. Exposure to a NOAC without a specific DDI was defined based on the treatment period of the NOAC. Index date was the first date of the NOAC treatment period for all patients. Individuals with an interacting drug treatment period that overlapped or started at the index date were considered exposed to the DDI. Exposure to a DDI ended at the last date of the treatment period of either the NOAC or the interacting drug, whichever occurred first.

5.6 DEFINITIONS OF COMORBIDITIES, COVARIABLES AND DESCRIPTIVE VARIABLES

The primary focus of studies I and II was the descriptive characterization of co-prescribed interacting drugs and the prevalence of these potential DDIs in the Swedish population. However, in both studies similar analyses of risk of exposure to DDIs were conducted in relation to age, sex and number of drugs. In study III, the change in warfarin dose was analyzed with age and sex as covariables. All covariables were treated as categorical variables. Age categories in study I were 0–5, 6–18, 19–64, 65–79 and ≥ 80 years of age. In study II, age groups were 0–2, 3–5, 6–11, and 12–17 years of age. In study III, the age groups were, 18–49, 50–59, 60–69, 70–79, 80–89 and 90–100 years of age. In study I, number of drugs were categorized as 2–4, 5–7, 8–10, 11–13, 14–16, 17–19 and ≥ 20 . In study II, the same categories were used for numbers below 11 drugs, whereas 11 or more drugs constituted the highest category.

Study IV in contrast, was more complex with regard to covariables due to the multiple relevant comorbidities potentially contributing to the outcomes. All variables were treated as categorical variables. Age, sex, and interacting drugs were included as descriptive variables and covariables in the analyses. For both purposes these variables were defined at baseline, i.e. the index date. Age groups were defined as <65 , 65–74, 75–79, and ≥ 80 years of age. Age groups were selected based on the CHA₂DS₂-VASc and HAS-BLED criteria with the addition of an older age group used for descriptive purposes but not for the covariable [130–132]. Interacting drugs were categorized into three groups as described above, pharmacodynamic interactions, drugs that interact by inhibition of CYP3A4 and/or P-gp, and drugs that interact by induction of CYP3A4 and/or P-gp. The drugs included in the respective group differed for each NOAC studied depending on the underlying evidence for the interactions based on Janusmed interactions and EHRA guidelines [5, 27].

The components of CHA₂DS₂-VASc and HAS-BLED were included in study IV as descriptive variables and covariables, with the exception of INR that could not be identified in the data sources and use of platelet aggregation inhibitors which was included among interacting drugs with pharmacodynamic effect [130–132]. In addition, mean CHA₂DS₂-VASc score was included as a descriptive variable. A number of additional comorbidities were included as descriptive variables to characterize the study population. All diagnose-based covariables and descriptive variables were identified in the Patient Register. No differentiation was applied regarding primary and secondary diagnoses. The choice of variables and specific ICD-diagnoses to include for each variable were pre-defined by discussion in the research group consisting of medical doctors specialized in clinical pharmacology and internal medicine/endocrinology, and by consultation of specialists in cardiology, neurology, and nephrology. Furthermore, publications from previous similar studies and the publications defining CHA₂DS₂-VASc and HAS-BLED criteria were

consulted [112, 130-135]. For descriptive variables and covariables, ICD-diagnoses representing each variable were identified before or at index date in the Patient Register. ICD-diagnoses were selected based on the highest level of truncation that included only specific diagnoses considered relevant for each variable. Three different time periods, ten years, five years, and six months were used for identification of diagnoses before index depending on the relevance of the diagnosis at index date. E.g. acute renal failure N17 was considered relevant if it occurred within six months before index date whereas chronic renal failure N18 was considered relevant if it occurred within ten years preceding index date. Both diagnoses were part of the variable “renal disease”. A detailed description of included ICD-diagnoses and the time periods considered for each diagnose can be found in supplementary table 1 of paper IV.

5.7 DEFINITIONS OF OUTCOMES

Studies I and II are descriptive to a large extent. The primary results are the descriptions of combinations of potentially interacting drugs that occur in the population. Frequencies of these potential DDIs at the time of the studies are given according to the DDI-groups based on potential clinical consequences and drug groups, as described above. In study I the risk of being exposed to a potential class D interaction was analyzed in relation to age, sex, and number of drugs. In study II a similar analysis was conducted but with exposure to a potential class C or D interaction as outcome. Furthermore, in both studies the frequency of the drugs most frequently involved in D-interactions was related to total dispensation of these drugs.

The primary endpoint of study III was the difference in mean warfarin dose between a four-week period 1 to 28 days before and a period 120 to 147 days after initiation of amiodarone treatment. The later time period was selected based on the descriptive analysis of weekly change in dose during concomitant treatment to reflect the time point when full effect of the interaction on dosing had occurred. Warfarin dose and INR were described for each week of the study period. Outcomes of these descriptive measures were weekly change in warfarin dose related to the baseline dose, weekly mean INR, and the proportion of patients with INR above 3 and 4, respectively.

Study IV had two composite primary endpoints. For interactions that potentially lead to increased risk of bleeding, the primary endpoint was any severe bleed (gastrointestinal bleeding, hemorrhagic stroke, other intracranial bleeding, other severe bleeding), whereas for interactions that potentially lead to reduced effect of the NOAC the primary outcome was ischemic stroke/stroke unspecified/TIA. Additionally, secondary endpoints for potential bleeding interactions were gastrointestinal bleeding and intracranial bleeding. Secondary endpoints for interactions that may lead to reduced effect of NOACs were ischemic stroke

and venous thrombosis. In contrast to descriptive variables and covariables both the Patient Register and the Cause of Death Register were used to identify diagnoses that constituted the endpoints [120, 127]. Outcome variables and relevant ICD-diagnoses included in each variable were selected and defined by the procedure described for descriptive variables and covariables above. However, diagnoses were only considered relevant if they occurred during the time of exposure. Diagnoses constituting primary cause of death as well as contributing causes were considered. Death was not an outcome (see censoring below), if the cause of death or a contributing cause was an outcome diagnosis, this was considered as an outcome.

5.8 STATISTICAL METHODS

In studies I and II the prevalence of at least one dispensed prescribed drug was calculated as well as prevalence of more than one dispensed prescribed drug. Based on the dataset, the prevalence of clinically relevant DDIs was calculated for the whole population, in different age groups, and for patients prescribed two drugs or more. The frequencies of potentially interacting drugs in studies I and II were calculated based on the number of combinations of these drugs in the respective study population at the time of the study period.

Consequently, one patient could contribute with more than one DDI to the summed frequencies. Additionally, the proportions of class A, B, C, and D interactions were calculated among concomitant dispensed prescribed drugs in study I. Furthermore, in both studies the proportions of potential DDIs that may lead to adverse events and reduced effect of treatment respectively were calculated. Substances involved in class D interactions were ranked according to the frequency of interactions and according to the frequency of interactions in relation to total dispensed prescriptions for each substance. The risk of being prescribed a D-interaction among patients dispensed at least two drugs was analyzed in relation to age, sex and number of drugs, with unconditional binary logistic regression. In study I the reference group was males, aged 19–64 years, dispensed two to four drugs. In study II the reference group was boys, 0–2 years, dispensed two to four drugs.

In study III, the primary endpoint, difference between mean baseline warfarin dose and mean dose at full interaction effect, was analyzed in a two-sided dependent t-test. Since a right-skewed distribution of mean dose for each patient around the mean of the population can be expected, log-transformation was conducted. Difference in log-transformed mean dose between the two time periods was calculated for each patient. The difference was then compared with zero, no difference, in the t-test, and confidence interval and p-value were calculated. The mean difference was then retransformed which provided a relative measure of the difference between the two time periods. Additionally, to provide a visual representation of change in mean weekly dose, the dose at week 18 to 21 was plotted against the dose during four weeks before initiation of amiodarone. In secondary analyses, the study population was divided based on baseline warfarin dose below 33 mg, and 33 mg or more per week. The

procedure described above for the primary analysis was then applied to these groups separately.

In another secondary analysis, the potential impact of age and sex on the association between amiodarone treatment and warfarin dose was investigated. The mean warfarin dose at baseline and mean dose at full interaction effect was calculated. Values were normalized in relation to the baseline dose and then log-transformed, and a multiple linear regression model was fitted that included categorical variables for age and sex. Log transformation was conducted since the distribution of relative doses are right skewed around the mean of the study population. The dependent variable was change in log-transformed normalized warfarin dose.

Weekly warfarin dose related to baseline dose and weekly INR was calculated for the study period. The warfarin doses were normalized by dividing the weekly dose to the baseline dose for each patient. The rationale for the normalization was the assumption that a potential interaction effect would be more uniform among patients in relative than in absolute numbers, i.e. a patient with a large baseline dose may have a larger absolute change in dose than a patient with a low baseline dose, but changes may be more similar in relative doses. Mean weekly warfarin doses and 95 % confidence intervals were calculated and doses were plotted against time during the study period. Additionally, the fractions of patients with > 10 %, > 25 %, or > 50 % reduction in weekly dose were calculated. The weekly dose was divided with the baseline dose for each week and patient, and fractions were calculated for each week and plotted against time. For weekly INR, the method proposed by Rosendaal et al was applied to account for missing data in weeks between measurements [136]. The time between two measurements of INR was divided in days and the cumulative change in INR was allocated in a linear fashion between these days. Subsequently, all values were log-transformed and weekly INR with 95 % confidence intervals were calculated. Finally, values were retransformed and plotted against time, to visualize the weekly mean INR during the study period. As for the distribution of weekly doses, log-transformation was conducted since the distribution of means of weekly INR for patients is right skewed around the mean of the study population. The fractions of patients with INR above 3 and 4 respectively were calculated for each week and plotted against time. Furthermore, the proportions of patients that had not had INR measured at 1, 2, and 3 weeks after initiation of amiodarone were calculated.

In study IV, primary and secondary analyses were conducted for each NOAC, i.e. no analysis was conducted for the whole group of NOACs. The rationale for this was that, though there are substantial overlaps between NOACs in mechanisms for interactions and interacting

drugs, there are also differences both regarding interacting drugs, mechanisms and clinical impact of interactions, see the section on mechanisms for interactions above. However, to achieve power in the analyses, interacting drugs were grouped according to mechanism of the interactions. Additionally, the number of patients with edoxaban and the number of patients exposed to edoxaban with interacting drugs in the population were too low to allow analyses. Consequentially, individual analyses were conducted for apixaban, rivaroxaban, and dabigatran in combination with groups of drugs with potential pharmacodynamic interactions, interactions by inhibition of CYP3A4 and/or P-gp, and interactions by induction of CYP3A4 and/or P-gp, respectively. Primary and secondary analyses were conducted with Cox-regression in multivariate survival analyses. Only patients treated with the analyzed NOAC were included in each analysis. Hazard ratios were calculated for patients exposed to each respective interacting drug group compared to patients without exposure to the group. Analyses of each mechanistic interacting drug group were adjusted for exposure at baseline to the other two groups. Analyses of interactions that potentially increase the risk of bleeding were adjusted for selected components of HAS-BLED, i.e. hypertension, renal disease, liver disease, ischemic stroke/stroke unspecified/TIA, any severe bleed, anemia, age category, and alcohol abuse [130, 132]. INR was not available and could not be included. Platelet aggregation inhibitors were included among drugs with pharmacodynamic effect. Analyses of interactions that potentially increase the risk of thromboembolism were adjusted for the components of CHA₂DS₂-VASc, i.e. heart failure, hypertension, diabetes, ischemic stroke/TIA/arterial embolus/stroke unspecified, vascular disease, age category, and sex [130, 131].

All individuals recorded in the Prescribed Drug Register for the study period, and fulfilling inclusion criteria and not exclusion criteria, were included in the analysis. However, power analyses were conducted prior to analyses to estimate the probability of successfully finding significant results if differences in risk existed between groups. The probability of successfully identifying a HR of 2 with 1000 patients exposed to an interaction was estimated at 83-89 %, depending on the NOAC analyzed. This was the reason for aggregating interacting drugs into groups. Censoring was performed at the end of exposure to NOAC or the interacting drug, or death. Migration could not be traced but was assumed to lead to end of exposure based on the Prescribed Drug Register. The proportional hazards assumption was evaluated by analysis of Schoenfeld residuals and exploratory Cox-regression analyses to evaluate covariate-time interactions were performed. Furthermore, evaluation of potential statistical interaction between covariates was conducted. Descriptive variables were presented with frequencies, percentages, and means.

Statistical analyses were performed in IBM SPSS Statistics 22.0 in study I, R version 3.0.2 in study II, IBM SPSS Statistics 22.0 and R version 2.0.3 in study III, and R version 3.6.1 in

study IV [137, 138]. Associations were presented with 95 % confidence intervals and statistical significance was defined at the 5 % level.

6 ETHICAL CONSIDERATIONS

All the studies in this thesis are retrospective and based on register data. In retrospective observational studies one of the major ethical concerns is personal integrity of the individuals that constitute the study population. General consideration of study conduct, and quality are other important aspects of the ethical evaluation.

Guidelines on research ethics were adopted in 1964 by the World Medical Association (WMA) in the Declaration of Helsinki. These guidelines are fundamental for medical research conducted on human subjects and have been updated continuously during the years [139]. The declaration is not legally binding but have had great influence on national legislation in many countries. One of the core principles of the declaration is that the rights and interests of individual research subjects always take precedence over scientific interests. Furthermore, the declaration stipulates that the privacy of research subjects and confidentiality of their personal information must be protected. In addition to the declaration of Helsinki, the Swedish Research Council has published the document “Good Research Practice” with guidelines on the conduct of research and research ethics [140]. A number of national laws are applicable to the conduct of medical research. Legislation applicable to the type of observational research conducted in the studies of this thesis are the act concerning the Ethical Review of Research Involving Humans (SFS 2003:460) and the General Data Protection Regulation (GDPR) of the European Union.

In studies I, II, and IV, all data were retrieved from registers maintained by the National Board of Health and Welfare. The identification of all data concerning each individual in the original datasets of the registers was based on the Swedish personal identity numbers, unique to each individual. However, data were delivered to the research group anonymized, and consequently no personal identity numbers were available to the group in any of these studies. In study III, data were linked between registers that are maintained by different organizations. Data from the warfarin monitoring registers Auricula and Journalia had to be linked to data from the Prescribed Drug Register by personal identity numbers. This inevitably entailed a slightly higher risk for research subjects from an integrity perspective than in the other studies. However, after the linking of register data it was anonymized, and personal identity numbers were not further used in the study. The above measures were taken to safeguard the personal identities of individual study subjects and minimize the spread of personal information not needed for implementation of the studies.

However, there are other ways of potentially identifying individuals in the datasets apart from through personal identity numbers. In all studies large amounts of data were retrieved concerning a large number of patients. Consequently, it may be possible to identify some

patients through the combination of drugs, diagnoses, dates for dispensations and diagnoses, age, and sex available in the datasets. Measures were therefore taken to store and handle datasets in such a way that the risk of spreading the data was minimized.

The publication of results also needs to be evaluated from the perspective of safeguarding the personal data of study subjects. In all papers of the thesis, results are presented in such a way that individuals cannot be identified in a meaningful way. Obviously, low frequencies of interacting combinations of drugs may be linked to individuals based on the time of study but doing so would require prior knowledge of the information and no further information can be linked to individual study subjects in the presentations of results.

An important aspect of research ethics is the potential benefit to study subjects of being included in the study. Regarding the studies of this thesis, as they were all retrospective and register based, there were no direct benefits for included patients. However, the results of the studies may contribute to safer drug treatment for future patients in general and therefore inclusion may indirectly benefit the study subjects.

A fundamental principle of the Helsinki declaration is that participation in medical research as a subject must be voluntary and that informed consent to participation need to be obtained. It is not uncommon though, that observational studies are granted exceptions from this principle if the risk to, or effect on, study subjects by the study is considered minimal and if the value of the research planned is assumed to outweigh the minimal risk to patients. Based on the above considerations, the risk to individual study subjects regarding their personal information was considered low in all studies and exceptions from the principle of informed consent were given by the Ethical Review Boards.

Medical research in general is conducted to gain knowledge and to contribute to improved medical practice in the future. Not only the patient risk, but financial resources, the time and dedication of researchers and collaborators, and the alternative cost of using resources that could be dedicated to research potentially more relevant are all examples of resources invested in a study. Furthermore, if the results are not good estimations of reality they may lead to false conclusions, and misguided practice in healthcare and further research. All aspects of design and implementation of a study are therefore relevant to evaluate from the perspective of research ethics. A few aspects of the studies in this thesis can be considered, that are further outlined in the discussion section, and that need to be weighed against the strengths of the studies.

The design of studies I and II, could potentially have been improved by a more precise identification of concomitant drug use. Furthermore, comparability with other studies would have been improved by a clear presentation of prevalence in paper I. However, the exposure to potential DDIs had not been described in the whole Swedish outpatient population with the high level of detail of these studies and no updated information was available at the time of publication.

Based on the previous research available, the contribution of study III may be questioned. But by increasing the precision of results compared to previous studies relevant, information was indeed added. Furthermore, since time in therapeutic range (TTR) and monitoring practices for warfarin treatment vary between populations the estimates may be more relevant to the conditions in Swedish healthcare and similar settings than results of previous studies.

The power analyses in study IV indicated that some of the analyses would not yield significant results. However, by analyzing all available data on Swedish atrial fibrillation outpatients dispensed NOACs, a contribution to the understanding of the impact on DDIs in this patient population was achieved based on the available data at the time. The use of NOACs is continuously increasing and it is highly relevant to conduct repeated observational studies during the lifetime of drugs to improve on the understanding of risks connected with their use.

All studies in the thesis were approved by the Regional ethics review board in Stockholm, Sweden.

Reference numbers:

Study I and II: 2008/1101-31/2

Study III: 2013/1008-31/3

Study IV: 2018/207-31

7 RESULTS

7.1 POTENTIAL DRUG-DRUG INTERACTIONS IN THE SWEDISH POPULATION

At the time of studies I and II, the Swedish population was 9 340 682 people, of which 1 921 093 people were 17 years of age or younger. The mean age in the whole population was 49 years, 50 % were women [141]. In the whole population, the mean (SD) number of dispensed prescription drugs was 3.8 (3.4). Half of the whole population and 27 % of children had at least one dispensed prescription drug, 35 % and 12 % were dispensed two or more drugs.

In table 5 prevalence estimates of clinically relevant potential DDIs in different age-groups are given for the whole population and the pediatric population. Additionally, in table 6 prevalence estimates are given for patients prescribed two or more drugs. In the whole population 6.4 % had at least one prescribed, dispensed, potentially clinically relevant DDI (class C or D), whereas among patients with two drugs or more 19 % had at least one potentially clinically relevant DDI. 2.5 % of patients with two drugs or more had a class D DDI. Among pediatric patients with two drugs or more, 1.4 % had at least one potentially clinically relevant DDI.

Table 5. Prevalence of DDIs, prescribed and dispensed in outpatient care, for different age groups in the whole population, January 1 - April 30, 2010.

DDI class	Age group							0 – 17	All ages
	0 – 2	3 – 5	6 – 11	12 – 17	18 – 64	65 – 79	≥80		
	n=333 152	n=320 652	n=588 201	n=679 088	n=5 728 812	n=1 196 392	n=494 385	n=1 921 093	n=9 340 682
C or D	0.05 %	0.11 %	0.13 %	0.29 %	4.1 %	18 %	30 %	0.17 %	6.4 %
C	0.05 %	0.11 %	0.13 %	0.27 %	3.8 %	17 %	28 %	0.16 %	6.1 %
D	0.005 %	0.005 %	0.005 %	0.04 %	0.55 %	2.5 %	4.1 %	0.02 %	0.88 %

Table 6. Prevalence of DDIs, prescribed and dispensed in outpatient care, for different age groups among patients with ≥ 2 dispensed prescription drugs, January 1 - April 30, 2010.

DDI class	Age group	
	0 – 17 n=2 31 078	All ages n=3 243 419
C or D	1.4 %	19 %
C	1.3 %	17 %
D	0.14 %	2.5 %

In the study population including all ages in study I, the total number of clinically relevant potential DDIs found (class C or D) was 1 048 165. Among these, 9 % were class D and 91 % class C interactions. Additionally, 1 058 860 class B interactions were found (clinical outcome of the interaction is uncertain and/or may vary). The inclusion of class A interactions (minor interaction of no clinical relevance) is not complete in the Janusmed interaction database, however 383 126 class A interactions were found in the study population. Note that in contrast to frequencies of exposed patients used for prevalence estimates, each patient could contribute with more than one DDI to these frequencies of potentially interacting drug combinations.

DDIs that potentially lead to reduced treatment effect constituted 49 % of class D interactions and 54 % of class C interactions in the whole population. In the pediatric population the corresponding proportions were 48 % and 32 %.

DDIs that may cause bleeding, cardiac arrhythmias, or serotonin toxicity were most common among adverse effect-type class D interactions in the whole population whereas reduced analgesic, anti-infective, or anticoagulant effect were most common among class D DDIs that may cause reduced treatment effect. In the pediatric subset of the population, serotonin toxicity and/or anticholinergic side effects and cardiac arrhythmias were the most common types of adverse effects associated with potential class D DDIs whereas the most common reduced effect-type DDIs concerned anti-infectives, opioids, and contraceptives.

Like for the class D interactions, combinations that may cause bleeding, cardiac arrhythmias, or serotonin toxicity were most common among class C interactions in the whole population. For interactions that may lead to reduced effect, interactions that may reduce the effect of drugs for hypertension, osteoporosis, and thyroid hormone substitution therapy were most

common. Among pediatric patients, class C interactions that may lead to toxicity from anticonvulsant or cytostatic drugs, and combinations that increase the risk of bleeding were most common. For reduced effect-type class C interactions, drug combinations that affect anticonvulsants and benzodiazepines were most common in the pediatric subset of the population.

Tables 7 and 8 describe the 10 most common drug combinations found in the whole population and the pediatric population, with potential class D and C interactions. In study I, the 15 most common drug combinations were found to constitute 80 % of the total frequency of interacting DDIs in the whole population. Furthermore, ten specific drugs were involved in 94 % of all class D interactions (table 9). The corresponding fraction for the ten drugs most frequently involved in class D interactions in the pediatric population was 78 %.

Table 7. The most 10 frequent potential D- and C-interactions, in the whole population, January 1 - April 30, 2010.

D-interactions		C-interactions	
Drug combination	Frequency	Drug combination	Frequency
Quinolone/tetracycline ¹ - metal ion	11 158	Diuretic ⁶ - NSAID/ASA	100 937
Potassium - potassium-sparing diuretics	9 902	Beta blocker - NSAID/ASA	80 296
Warfarin - ASA	9 523	Acetylsalicylic acid - antidepressant ⁷	79 331
Proton pump inhibitor ² - clopidogrel	9 042	Metal ion - bisphosphonate	61 308
Calcium antagonist ³ - beta blocker	6 985	NSAID - antidepressant ⁷	53 942
Tramadol - antidepressant ⁴	6 439	ACE inhibitor - NSAID/ASA	49 830
Codeine/ethylmorphine - antidepressant ⁵	5 415	ARB - NSAID/ASA	41 778
Warfarin - NSAID	4 512	Levothyroxine - metal ion	40 823
Verapamil - digoxin	2 052	Potassium sparing diuretic - ACE inhibitor	40 802
Diazepam - carbamazepine	1 869	Paracetamol - warfarin	30 741

¹ Ciprofloxacin, doxycycline, lymecycline, norfloxacin and tetracycline.

² Omeprazole and esomeprazole.

³ Diltiazem and verapamil.

⁴ Bupropion, duloxetine, fluoxetine, clomipramine and paroxetine.

⁵ Bupropion, fluoxetine and paroxetine.

⁶ Amiloride, bendroflumethiazide, bumetanide, eplerenone, furosemide, hydrochlorothiazide, spironolactone and torasemide.

⁷ Citalopram, duloxetine, escitalopram, fluoxetine, fluvoxamine, clomipramine, paroxetine, sertraline and venlafaxine.

Table 8. The most 10 frequent potential D- and C-interactions, in the pediatric population, January 1 - April 30, 2010.

D-interactions		C-interactions	
Drug combination	Frequency	Drug combination	Frequency
SSRI - SSRI ¹	84	Valproic acid - lamotrigine	539
Quinolone/tetracycline ² - metal ion	69	Mesalazine - azathioprine	259
Potassium - spironolactone/amiloride	19	Salbutamol/terbutaline - ipratropium	192
Ethylmorphine - fluoxetine	18	NSAID - antidepressant ⁵	179
Oxcarbazepine - gestagen/estrogen ³	17	Carbamazepine/phenytoin - diazepam/midazolam	164
Carbamazepine - gestagen/estrogen ⁴	15	Methotrexate - beta lactam antibiotic ⁶	135
Warfarin - ASA low dose	14	Valproic acid - topiramate	108
Codeine/tramadol - fluoxetine/paroxetine	14	Levothyroxine - metal ion/sucralfate	106
Diazepam - carbamazepine	13	Trimethoprim/sulfamethoxazole - methotrexate	102
Risperidone - carbamazepine	8	Beta blocker - NSAID/ASA	95

¹ Sertraline, fluoxetine, paroxetine, escitalopram, citalopram.

² Ciprofloxacin, doxycycline, lymecycline, norfloxacin, tetracycline.

³ Medroxyprogesterone, desogestrel, ethinylestradiol, drospirenone, etonogestrel, norethisterone, levonorgestrel, norelgestromin.

⁴ Medroxyprogesterone, ethinylestradiol, desogestrel, norethisterone, levonorgestrel, norelgestromin.

⁵ Fluoxetine, citalopram, escitalopram, sertraline, paroxetine, duloxetine, venlafaxine, fluvoxamine.

⁶ Phenoxymethylpenicillin, amoxicillin, flucloxacillin, pivmecillinam.

Table 9. The ten substances most frequently involved in potential D-DDIs in the whole population with percentages of the total frequency of potential D-DDIs.

Substance	Frequency	% of D-DDIs
Warfarin	17 918	19 %
Clopidogrel	9 922	11 %
Potassium	9 902	11 %
Acetylsalicylic acid	9 822	10 %
Carbamazepine	8 084	9 %
Omeprazole	7 715	8 %
Ciprofloxacin	7 102	8 %
Tramadol	6 743	7 %
Fluoxetine	6 020	6 %
Amiloride	5 782	6 %
Sum	89 010	94 %

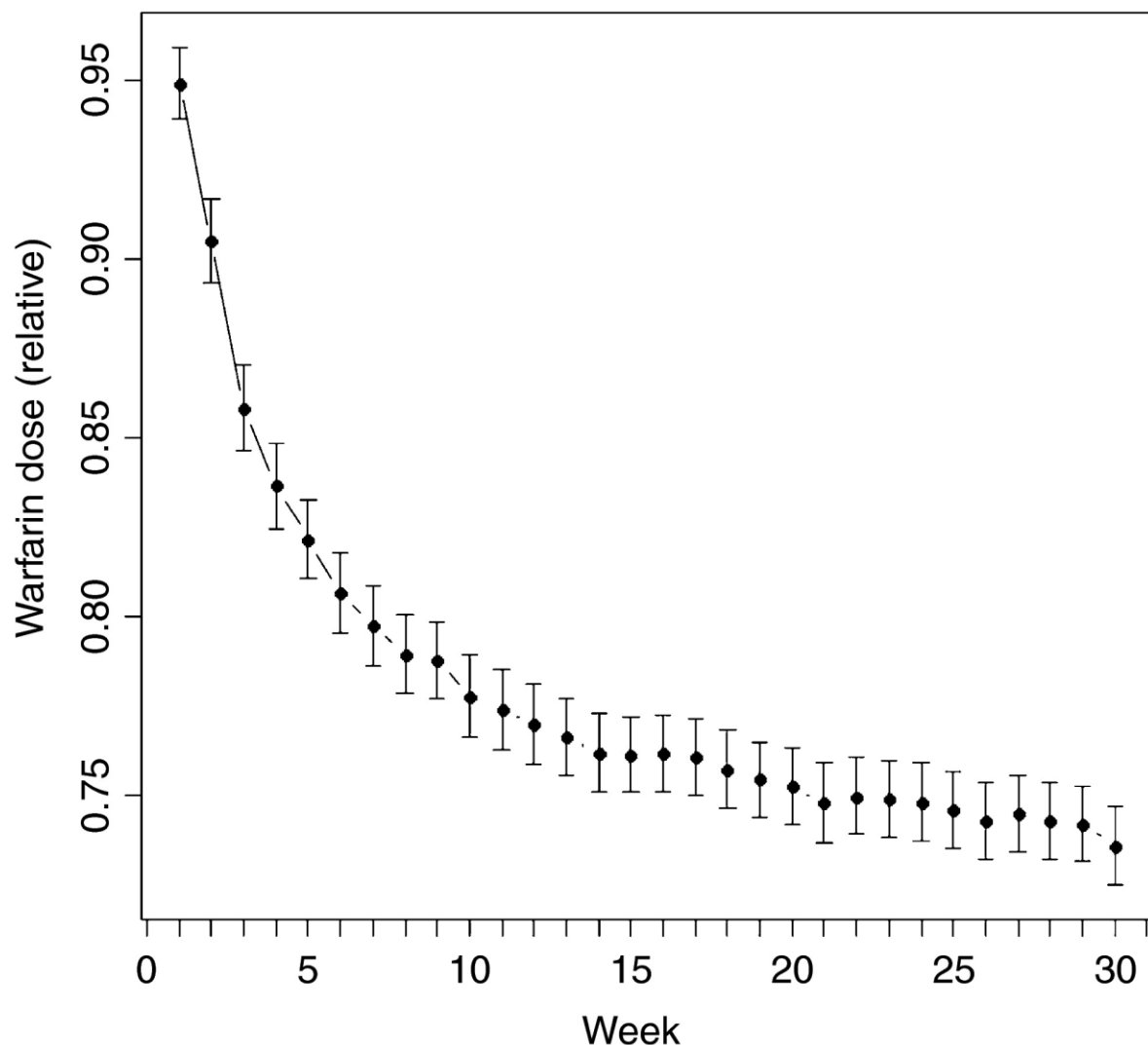
The effect of age, sex and number of drugs on the risk of exposure to DDIs was investigated in the whole population and in the pediatric subset of the population. In the analysis of whole population, the reference group was men aged 19-64 years with two to four drugs whereas in the pediatric subset it was boys aged 0-2 years with two to four drugs. For the whole population the association with the risk of being exposed to at least one potential D-interaction was analyzed. Adjusted OR (95 % CI) for increasing number of drugs ranged from 5.7 (5.5–5.8) for five to seven drugs, to 111 (107–116) for ≥ 20 drugs, compared to the reference population. The younger and older age groups had a decreased association, 0-5 years 0.039 (0.027–0.055), 6–18 years 0.29 (0.26–0.32), 65–79 years 0.95 (0.93–0.96), and ≥ 80 years: 0.83 (0.81–0.84). Furthermore, women had a decreased association 0.79 (0.78–0.80). In the pediatric subset in contrast, the association with exposure to at least one potential class C or D interaction was analyzed. Adjusted OR (95 % CI) increased with increasing age, 3-5 years 2.10 (1.74, 2.53), 6-11 years 3.74 (3.16, 4.45), and 12-17 years 6.89 (5.88, 8.13). For increasing number of drugs, odds ratios were increasing 5-7 drugs 6.46 (5.95, 7.00), 8-10 drugs 21.14 (18.78, 23.75), and ≥ 11 drugs 59.65 (50.90, 69.79). Girls had a slightly higher adjusted OR, 1.10 (1.02, 1.18).

7.2 EFFECT OF AMIODARONE ON WARFARIN ANTICOAGULATION

In study III, 754 unique individuals were identified that fulfilled inclusion criteria and who did not fulfill exclusion criteria. The median age was 67 years (range 23 - 90 years) and 30.5

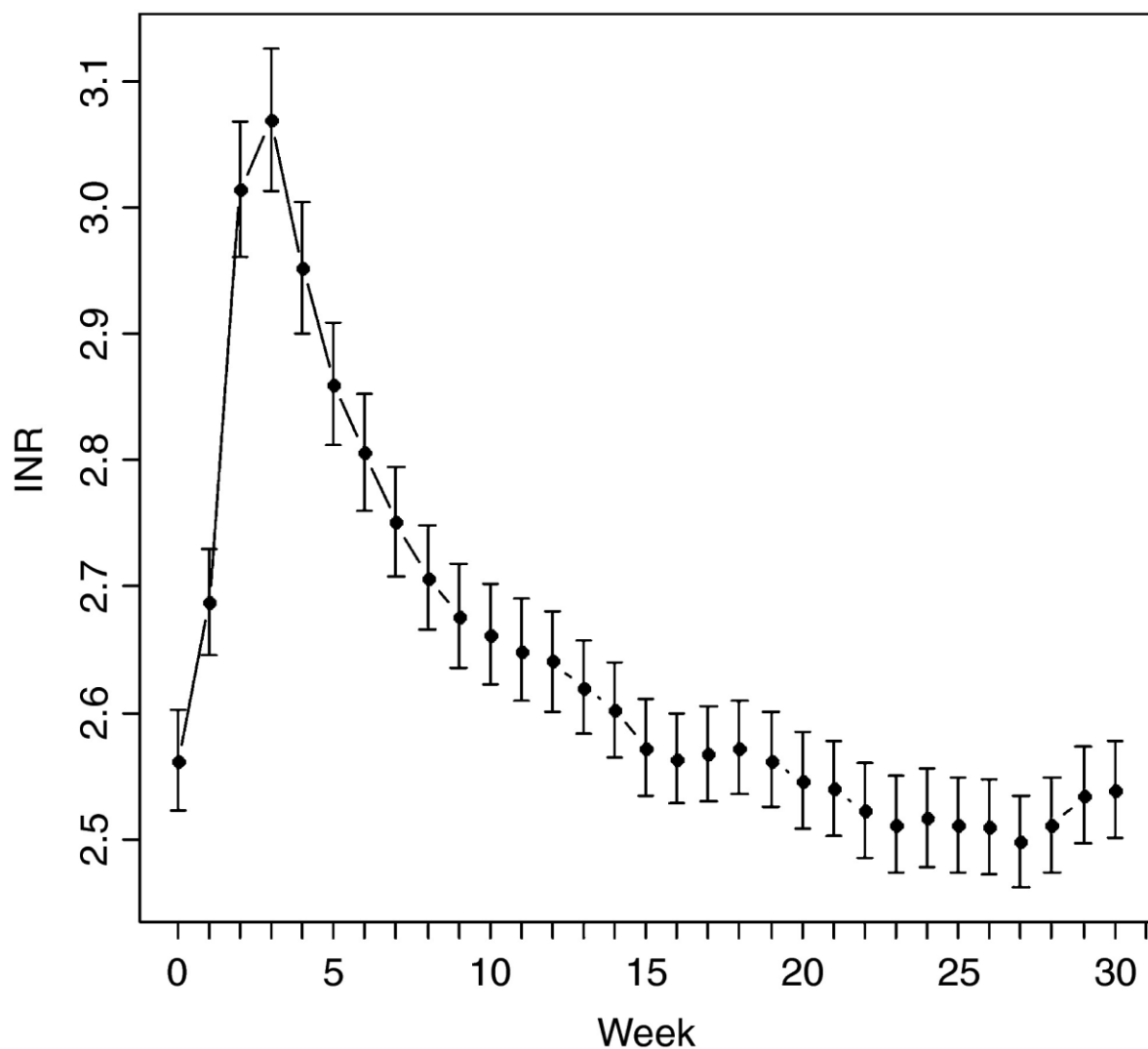
% were women. Mean baseline weekly warfarin dose was 34.6 mg. A four-week period at 120-147 days was identified when full effect of concomitant treatment with amiodarone had developed on warfarin dose (figure 5). During these four weeks, the mean weekly warfarin was 24.6 % (95 % CI, 23.5–25.6 %; $P < 0.001$) lower than the weekly baseline dose. There was no difference between men and women or in different age groups in change of warfarin dose between baseline and this four-week period during concomitant treatment with amiodarone. The majority of patients had a reduced dose of between 0 and 50 % compared to baseline at the time of this four-week period. Furthermore, the majority of patients (87.1 %) had a dose reduction of at least 10 %, many (55.1 %) had a dose reduction of more than 25 %, whereas a smaller number (3.1 %) had a dose reduction of more than 50 %. Patients with baseline warfarin dose of 33 mg or more had a dose reduction of 25.5 % (95 % CI, 24.1–26.8 %), whereas patients with a baseline dose below 33 mg had a dose reduction of 23.7 % (95 % CI, 22.1–25.3 %).

Figure 5. Mean weekly warfarin dose in relation to baseline dose after initiation of amiodarone treatment in patients treated with warfarin (means and 95 % confidence intervals). Reproduced with permission, Holm et al 2017.



Mean weekly INR increased after initiation of amiodarone and peaked at 3.07 (95 % CI, 3.01–3.13), the third week of concomitant treatment. After about 25 weeks it returned to baseline after gradual decrease (figure 6). At index date the fraction of patients with an INR above 3 was 11.7 % and at the third week of concomitant treatment this number had increased to 37.1 %. This fraction returned to baseline after about 20 weeks of concomitant treatment. With a similar pattern, 0.9 % of patients had INR over 4 at baseline, this fraction increased to 5.5 % at the third week of concomitant treatment. At week 12 it returned to the baseline level. 67 % of patients had INR measured within one week of concomitant treatment with amiodarone, whereas after two and three weeks the fractions were 90 % and 96 % respectively.

Figure 6. Mean weekly INR each week after initiation of amiodarone treatment in patients treated with warfarin (means and 95 % confidence intervals). Reproduced with permission, Holm et al 2017.



7.3 CLINICAL EFFECT OF DRUG-DRUG INTERACTIONS IN NOAC TREATMENT

In study IV, 244 597 patients with presumed atrial fibrillation were identified that had new episodes of NOAC treatment between 2008 and 2017. The majority of these patients were prescribed apixaban (61 %), whereas rivaroxaban (24 %), dabigatran (15 %) were less common. Edoxaban was only used by a very small number of patients (<1 %) and consequentially it could not be included in analyses. Concomitant drug treatment that may lead to pharmacodynamic interactions was found in 48 % of patients. In contrast, potential pharmacokinetic interactions were less frequent, 4 % and 1 % for inhibitors and inducers of CYP3A4 and/or P-gp, respectively. Furthermore, a majority of patients treated with drugs that may lead to pharmacokinetic interactions also had drugs that may lead to pharmacodynamic interactions. Generally, patients who were exposed to potential DDIs also had more comorbidities and higher mean CHA₂DS₂-VASc score at baseline than patients without interacting drugs.

The association of exposure to potential pharmacodynamic DDIs with the primary endpoint, any severe bleed, was increased compared to patients not exposed to potential pharmacodynamic DDIs, apixaban HR (95 % CI) 1.47 (1.33-1.63), rivaroxaban 1.7 (1.49-1.92), and dabigatran 1.26 (1.05-1.52). For patients treated with apixaban, there was an increased risk of any severe bleed associated with exposure to CYP3A4 and/or P-gp inhibitors, 1.23 (1.01-1.5), but with a confidence interval approaching one. No significant effect could be established for patients treated with rivaroxaban 1.24 (0.94-1.65) or dabigatran 0.84 (0.48-1.45). No significant effects could be established for patients exposed to inducers of CYP3A4 and/or P-gp.

Exposure to pharmacodynamic DDIs was associated with increased risk of the secondary endpoint gastrointestinal bleed for patients with apixaban 1.51 (1.28-1.78) and rivaroxaban 1.68 (1.37-2.05). Additionally, exposure to pharmacodynamic DDIs was associated with increased risk of intracranial bleeding for rivaroxaban, though with a confidence interval approaching one, 1.44 (1.02-2.04). Similarly, exposure to inhibitors of CYP3A4 and/or P-gp was associated with increased risk of gastrointestinal bleeding, but with confidence intervals approaching one, for apixaban 1.44 (1.06-1.95) and rivaroxaban 1.54 (1.02-2.32).

A significant effect of time on the HR was detected for the analysis of the primary endpoint in patients with apixaban and inhibitors of CYP3A4. There was an indication that the effect may be larger towards the end of the study period according to this analysis. Statistical interactions between covariables were evaluated for primary endpoints in the apixaban dataset. No significant statistical interactions were found in this evaluation.

8 DISCUSSION

8.1 RESULTS DISCUSSION

8.1.1 Prevalence and exposure to potentially interacting drugs

Prevalence and frequencies of DDIs have been studied in various settings and with heterogeneous methodologies [82, 83]. Consequentially, reported estimates of prevalence and frequencies of interacting drugs need to be understood considering the study population and methodology used. However, similarities between studies allow for a general interpretation of the field and similarities in findings are not uncommon. Large cross-sectional studies are often conducted in drug registers, in some cases covering national populations [87, 142]. Other studies are aimed at specific hospital populations [143-145] or outpatients in primary healthcare [146]. Several studies have been conducted in Sweden and the other Nordic countries [87, 142, 147-149], describing populations that are more similar to the Swedish population than studies conducted in other parts of the world [143-145]. Many studies focus on elderly populations [89, 149-151], that are often exposed to more drugs than younger patients and increasing age have been associated with a higher probability for DDIs [144, 145, 148, 152-155]. A meta-analysis published in 2018 found prevalence estimates of exposure to potential DDIs in hospitalized patients ranging between 16.3 and 71.1 % [82]. Most included studies were from developing countries and therefore results may not be directly comparable to the Swedish setting. Another meta-analysis, published 2012, found prevalence estimates between 15 and 45 % of hospitalized patients [83]. A wide range of factors may contribute to the different estimates. Some, e.g. the different health profiles of populations studied or different age-ranges are factors that are relevant for the understanding of the estimated prevalence, whereas factors related to heterogeneity of methodology e.g. methods for identification of potential DDIs or the definition of the population at risk, are factors that make comparisons more difficult and complicate understanding of the actual exposure in different patient groups.

A number of observational studies on prevalence of DDIs have been conducted in Sweden apart from study I, most of them in elderly patients [80, 87-89, 146, 150, 156-158]. Only one of these studies was a nationwide study of all ages. It was a cross-sectional study based on a one-month dataset of the whole population from 1999 [87]. In the study including the whole population, prescriptions with two or more drugs were evaluated for DDIs. Class D interactions were found in 1.4 % and class C interactions in 8.1 % of those prescriptions [87]. The results are not comparable to the results of study I since only prescriptions with two drugs or more were included. We did not relate the number of interactions found to the number of prescription drugs dispensed. Furthermore, the study period was one month only, since drugs used at the same time by a patient may be prescribed and dispensed at different times during a longer time period than a month, the result from this study is more of an evaluation of the prescribing behavior of individual prescribers than of prevalence or

frequencies of interacting drugs in the population. However, not unexpectedly and as in study I, an increased risk of exposure to DDIs was found with increasing number of drugs. Increasing age was also associated with an increased risk of exposure to DDIs. In study I this was not the case, modest decreased risk was seen in the older age groups. In a cohort study based on patients from 20 primary healthcare centers the methodology was more similar to study I and II [158]. Dispensed prescription drugs representing concomitant drug treatment was identified in the Prescribed Drug Register for two four-month periods in the fall 2006 and spring 2007, to account for seasonal variation in drug prescribing. 27 543 class C interactions and 3025 class D interactions were identified in data from 125 287 patients, no prevalence of DDIs in the population was calculated. However, the numbers of potential class C and D interactions found in relation to the number of patients in study I were larger than interactions found in this study restricted to primary healthcare patients. A comparison of the frequencies of interactions is difficult since, because of different objectives, the frequencies of the study in primary healthcare summed potential DDIs from two time periods. One interacting combination from a single patient could be counted twice. A probable reason for the difference in frequencies of potential DDIs between the two studies could be selection of relatively healthier primary healthcare patients in this study compared to the patients in study I. Like study I, the most common class D interactions were combination of tetracyclines with chelate-forming drugs and potassium prescribed with potassium-sparing drugs, and for class C interactions combinations of NSAIDs and antihypertensives or diuretics. In general, the two studies have similar results regarding the type of DDIs found.

There are many studies internationally that investigate potential DDIs in various settings and estimate prevalence based on different definitions of populations at risk and methods for identifying interactions. Most of them are conducted in different hospital settings and relatively few are based on outpatients in general, including primary healthcare, in European populations [10, 82, 83, 147, 159-161]. For the purpose of comparison and to exemplify similarities and differences in results and methodology, two studies conducted in European outpatient populations will be discussed here. In a study based on the population in the County of Funen in Denmark potential DDIs were identified in purchased prescription drugs during 1999 [147]. One third of the population had been prescribed more than one drug. Prevalences of 11.2 % for moderate and 1.9 % for major interactions were calculated for this group, according to the classification of Hansten and Horn [162]. Notably, only the most significant interaction was counted for each patient. Compared to study I, conducted in Sweden in 2010, prevalence of more than one drug was roughly the same in this study. Prevalence of clinically relevant potential interactions was higher in study I, but the interaction databases and definitions of interaction classes are not identical. Additionally, the Danish study was conducted on a dataset based on a whole year and it could be argued that the prevalence calculated represents a period prevalence and that study I represents a point prevalence. In a study conducted in Slovenia in 2015, based on a nationwide database of drug prescriptions in outpatients, an annual prevalence of 9.3 % of clinically relevant potential

DDIs was calculated [163]. The population at risk for this estimate was defined as the whole Slovenian population and clinically relevant interactions were defined as drug combinations that should be avoided or drug combinations for which therapy modification should be considered according to the Lexi-Interact Module. These categories are roughly but not directly comparable to the definitions of type C and D interactions in the Janusmed interaction database. In study I the combined prevalence of either of these two types of interactions in the whole population was 6.4 %. Among patients with at least two dispensed drugs, with at least one of the drugs being the object of a potential interaction in the database, the prevalence of drug combinations for which therapy modification should be considered was 14.8 % and the prevalence of drug combinations that should be avoided was 2.9 % in the Slovenian study. These estimates are not very unlike the estimation of prevalence of class C and D interactions among patients with at least two drugs in study I but notably, the definitions of population at risk are not identical between the studies. The prevalence of clinically relevant potential DDIs seems to have been slightly higher in the Slovenian population in 2015 than in the Swedish population of 2010. Among patients exposed to two drugs or more, estimates of prevalence are more similar. Like the Danish study above, the Slovenian study was conducted on a dataset based on a whole year and estimates represent a period prevalence in contrast to study I. Additionally, for all three studies comparisons made between studies that use different interaction databases at different points in time should be interpreted with caution. Interaction databases develop constantly, including increasing number of drug combinations for new and older drugs, and it is not unlikely that more potential DDIs may have been detected should the analysis in older studies have been rerun with a newer version of the database used.

Many studies indicate that the risk of exposure to DDIs increase with age [144, 145, 152-155] and increasing number of drugs [89, 144, 145, 153-155, 164]. Furthermore, in a Dutch population-based cohort study prevalence of DDIs in people aged 70 years or older increased from 10.5 % to 19.2 % between 1992 and 2005 [165]. Additionally, a large number of studies focus on DDIs in the elderly [83, 89, 157, 166-169]. Notably, in study I we did not detect an increased association of exposure to DDIs with age. This result can be questioned in light of results from other studies indicating an increased risk of exposure to DDIs with increasing age. Furthermore, a Swedish cross-sectional study from 2007 calculated a prevalence of 5 % for class D interactions, and 26 % for class C interactions, according to the Janusmed classification, among patients aged 75 years or more with at least two drugs [89]. In this study, exposure to drugs was calculated based on prescribed dose in data from the Prescribed Drug register and overlapping drug treatment at one arbitrarily chosen date, 31 December 2005, was assessed for interactions. Patients with at least two drugs on this date were considered the population at risk. In study I, we did not calculate the specific prevalence for patients with at least two drugs this age group. However, compared to the prevalence in the whole population these results indicate a higher prevalence for the elderly. Notably, in study I a three-month period was used to estimate point prevalence. This could lead to a higher

estimation of concomitantly used drugs than the method used by the authors of the study in data from 2005. Similar estimates of prevalence were also found in an older Swedish study from 1995 [150]. In patients aged 78.2 years on average and included in a pharmacy drug dispensing program that supported outpatients with poor compliance, 3 % had DDIs of major clinical significance, and 23 % had DDIs of moderate clinical significance according to the classification in Hansten and Horn [162]. A different method of studying prevalence of DDIs among patients ≥ 65 years old that used ≥ 4 prescribed drugs was used in a study from 2002 [151]. This definition of the population at risk could be expected to result in higher prevalence estimates than in the studies discussed above. Cross-sectional data from a longitudinal multicenter study conducted in community pharmacies in Sweden, Denmark, the Netherlands, Portugal, the Republic of Ireland, Northern Ireland, and Germany were used, 1601 patients were studied. Compared to most population-based studies on DDIs the number of patients, though recruited from a large geographical area, was relatively small. The overall prevalence of any DDIs in this population of was 46 %. Among the interactions detected almost 10 % of the DDIs were class D and approximately 90 % were class C interactions, according to the Janusmed classification. 50 % of the interacting combinations could result in an adverse drug reaction and 50 % in a reduced therapeutic effect. In a study conducted 2007, of residents in assisted living facilities in Finland with a mean age of 82.7 years, a DDI prevalence of 5.9 % was seen for class D interactions according to the classification used in Janusmed interactions [148]. Like the Swedish study from 2005 described above, a point prevalence at one day was calculated. Another estimation of prevalence of DDIs during one month in nursing homes in Finland from 2008 found a similar prevalence of D-type DDIs of 4.8 % according to the Janusmed classification [84].

Drugs most frequently involved in potential DDIs in observation studies vary depending on when and where studies were performed, this is not unexpected since the drugs most frequently used vary over time and depend on the diseases common in different parts of the world. Furthermore, though ideally interaction databases should report the same interactions as clinically relevant, and do to a large extent, differences exist and may translate into different results in observational studies [170]. The focus of this text will not be a detailed description of similarities and differences, and comprehensive lists of frequent interactions can be found in papers I and II. However, many studies report overlapping drugs and drug combinations as the most frequent, and these drugs are found in study I as well. Examples of drugs and drug groups frequently involved in clinically relevant potential DDIs found in clinical practice are, in alphabetical order, ACE inhibitors, acetylsalicylic acid, anticoagulants, beta blockers, calcium antagonists, carbamazepine, codeine, drugs containing metal ions, NSAIDs, potassium supplements, potassium-sparing diuretics, quinolones, SSRIs, tetracyclines, and tramadol [84, 147, 148, 152, 158, 163, 171].

The risk of adverse drug reactions has been shown to increase with an increasing number of drugs among pediatric patients and off-label use of drugs is common in pediatric populations which may increase risks with DDIs [172-175]. The literature on potential DDIs in pediatric populations is limited though, and more often restricted to specific patient populations than focusing on outpatients in general [95-98, 176-178]. However, a few studies on DDI prevalence in children have been published. These studies have been conducted in different settings and populations. A retrospective cohort study conducted in 2011 investigated prevalence of potential DDIs in 498 956 hospitalizations of patients, 21 years of age or younger, at Children's Hospitals in the USA [93]. A contraindicated potential DDI was found in 5 %, a major DDI in 41 %, and a moderate DDI in 28 % of hospitalizations. The Micromedex Drug-Reax classification was used to identify DDIs. The most common drug classes were opioids (25 % of DDIs), anti-infective agents (17 %), neurologic agents (15 %), gastrointestinal agents (13 %), and cardiovascular agents (13 %). Among adverse events that may result from the interacting drug combinations, respiratory depression, increased risk of bleeding, QT interval prolongation, reduced iron absorption, central nervous system depression, hyperkalemia, and altered diuretic effect were common. The percentages of hospitalizations cannot be directly compared to the prevalence of potential DDIs found in study II and it is not unexpected that prevalence in outpatients exposed to more than two drugs would be considerably lower than fraction of hospitalized patients exposed to potential DDIs. The specific DDIs frequent in the two populations also differed considerably which is not unexpected comparing outpatients and hospitalized patients. In another study conducted at a university hospital in the Czech Republic, prevalence of DDIs was studied in 6078 patients, 19 years of age or younger, during a 12-month period in 2010 [91]. The Infopharm Drug Interactions Compendium was utilized to identify interactions. Potential DDIs were found in 3.83 % of patients only, and DDIs classified as moderate or severe in 0.47 %. The risk of being exposed to potential DDIs was associated with patient age, the average number of prescriptions per visit, and number of visits. Diagnoses of epilepsy, leukemia, or rheumatoid arthritis were associated with higher risk of DDIs. The difference in patients exposed to potential DDIs compared to the American study discussed above is remarkable. Furthermore, it is a small estimate compared to prevalence among pediatric patients with at least two drugs in study II. The authors call for caution in the interpretation of the results and acknowledge that the potential interactions found were few, but no further evaluation of this is offered. A comparison of comprehensiveness of the interaction database used in relation to other sources would have been a first informative step of evaluation, but none can be found in the available literature. As an example of a study conducted in a very different setting from the Swedish, one conducted in Nigeria can be mentioned [94]. Potential DDIs in prescriptions to pediatric outpatients were investigated and 16.9 % of the patients received prescriptions with DDIs during the 3-month study period. In this setting, it is not surprising that antimalarial therapies were dominating among drugs involved in DDIs and this exemplifies very clearly how differences in results depend on the setting of the study. An interesting aspect of their findings is that a correlation was seen between the severity of DDIs and the age of the patient. Apart from the studies discussed above, there are a number of studies on

prevalence of DDIs in different specialties. Studies in intensive care units describe populations with a high number of drugs per patient and, not surprisingly, much higher frequencies of DDIs than described in the studies in general populations above [95, 179]. Other authors describe prevalence of DDIs in pediatric oncology [96, 97] or epilepsy care [98, 99].

8.1.2 The effect of amiodarone on warfarin anticoagulation

Though the use of warfarin have declined since the NOACs emerged on the market, it is currently used by a significant number of patients [75]. Concomitant use of warfarin and amiodarone has been associated with an increased risk of bleeding-related hospitalizations with a risk ratio of 3.33 (95 % CI 1.38–8.00) compared to warfarin alone [103]. As previously mentioned in the objectives, the characterization of the effects on warfarin dose and anticoagulant effect measured by INR relied on data from small longitudinal studies and study III was conducted in a large dataset to increase the precision of estimates [52, 104-106]. Furthermore, since safe and effective warfarin treatment rely on TTR, that vary between different countries, and since pharmacogenetic profiles relevant to warfarin dose requirements differ between populations, studies in different settings provide broader understanding of the impact of the interaction [180, 181]. Previous studies reported slightly different estimates of decreased warfarin dose requirements after initiation of amiodarone. The results from study III are similar to results from a number of studies but differ compared to other [52, 102, 105, 106, 182, 183]. In a retrospective cohort study including 73 patients with INR outside the therapeutic interval, mean warfarin dose change to maintain an INR within the therapeutic interval was 25.6 % with a range of 5,9 to 65 % [102]. Similarly, in a study aimed at developing a pharmacogenetic dosing algorithm for warfarin, a 22 % warfarin dose reduction was needed in 36 patients that were using amiodarone and warfarin concomitantly [182]. A more marked mean dose reduction of 44 % was needed in a prospective cohort study of initiation of amiodarone in 43 patients on stable warfarin treatment [105]. The effect of the interaction peaked at seven weeks. Efforts were specifically made to keep INR within the therapeutic range and consequently results regarding this aspect may not reflect the situation in a clinical setting. The markedly higher estimate in this study may be the result of an initial loading dose of amiodarone used. That such an effect may be relevant is supported by studies that have shown an inverse correlation between warfarin maintenance dose and amiodarone dose [52, 105]. In a smaller study of only eight patients starting amiodaron treatment while having a stable prothrombin time during warfarin treatment, a mean 35 % reduction in daily warfarin dose, range 25 to 50 %, was needed to achieve baseline prothrombin time [106]. In a larger retrospective study of the impact of genetic variations and interacting drugs on warfarin maintenance dose in 845 patients, 87 patients had concomitant treatment with amiodarone that resulted in roughly 20 % reduction in mean warfarin maintenance dose [184]. Due to the inverse correlation between amiodarone dose and warfarin dose mentioned above, variations in amiodarone dose, especially in smaller

studies, could explain the differences in the estimates of mean warfarin dose reduction needed to compensate for the interaction effect. Furthermore, in study III and in the studies described above considerable variation in range of the doses needed to maintain INR within the therapeutic interval was seen. Warfarin dose reductions between 25 and 65 % before initiation of amiodarone treatment have been suggested [52, 105, 106]. A pre-emptive dose reduction of 25 % may be considered based on the results of study III and other studies, but with the interindividual variability in mind this should be considered with caution. However, due to the large interindividual variability in dose requirements during concomitant treatment close monitoring of INR is necessary, particularly during the first weeks when the effect seems to be largest before compensatory dose adjustment. In study I, the fraction of patients who had their INR measured during the first two weeks indicated a relatively high degree of monitoring but that some prescribers may not be aware of the interaction.

In study III, warfarin dose was markedly reduced during the first few weeks after which continued gradual reduction was seen during study period. A marked increase in mean INR to levels above the therapeutic interval was seen during the first few weeks, with a peak at 3 weeks after initiation of amiodarone treatment and subsequently gradually declining. In the cohort of 43 patients mentioned above, warfarin dose decreased gradually during the first seven weeks with the largest decrease during the first two weeks. After week seven, it increased gradually during the follow-up period of 12 months [105]. Similar dynamics were seen during the first weeks in subset of 52 patients from a larger cohort, for which warfarin and INR were followed for up to a year [104]. Warfarin dose decreased markedly during the first week and then gradually decreased until the sixth week after which it gradually increased again during the 12 months follow-up. It is possible that the increase seen in the later stages of follow-up in these two studies depends on decreased amiodarone doses during long-term therapy [105]. Furthermore, the continued decrease in doses during the study period of 30 weeks in study III may be related to a delayed response to the initial marked increase in INR. The fraction of patients with INR above 3 peaked at the third week and was increased during the first 10 to 12 weeks of concomitant treatment in study III. In the follow up of the 52 patients mentioned above, an INR above 5 was most common during the first 12 weeks of concomitant warfarin and amiodarone treatment. There were no peaks in the rate of INRs above 5 after 12 weeks of concomitant treatment and during the rest of the 80-week study period [104].

8.1.3 The clinical impact of interactions for patients treated with NOACs

Though the use of NOACs have increased markedly during the past years in Sweden and internationally, few observational studies can be found on the overall clinical effects of DDIs with NOACs [75, 107, 108, 112, 113, 185]. Only two studies with a general approach similar to study IV have been found [112, 113]. All outpatients prescribed and dispensed NOACs in

Sweden who fulfilled inclusion criteria and not exclusion criteria were included in study IV. This resulted in a large cohort of patients treated at different times during the majority of years that NOACs have been used in Sweden. Pharmacodynamic interactions were frequent in the cohort whereas pharmacokinetic interactions were less common. Consequentially, the results reflect the power to detect differences in treatment outcomes between the different groups of interacting drugs studied.

Patients treated with NOACs and drugs with pharmacodynamic effect on bleeding had a higher risk of bleeding events compared to patients without this group of interacting drugs. For combinations with pharmacodynamic effect, patients treated with apixaban and rivaroxaban had a higher HR of any severe bleed than patients treated with dabigatran. Furthermore, the lower bound of the confidence interval approached one for dabigatran. A similar pattern could be seen in secondary endpoints among patients treated with drugs that have a pharmacodynamic effect on bleeding. However, it is important to note that confidence intervals overlap, and that we did not formally compare different NOACs in the analysis. A case-control study of British primary care patients recently published also found increased risks for major bleeding events in patients treated with any NOAC and drugs with potential additive effect on bleeding [113]. However, in their analysis significant increased risks were seen for apixaban and dabigatran but not for rivaroxaban. These differences in results may depend on the distribution of different drugs, with different effects on the bleeding risk, within the group of drugs that interact pharmacodynamically. It is possible though, that there is a difference between the different NOACs in the clinical impact of pharmacodynamic interactions and this would warrant further investigation. Regarding individual drugs included in the group of pharmacodynamically interacting drugs, frequencies varied greatly. ASA was very frequent, clopidogrel and some of the NSAIDs fairly frequent and other drugs were less frequent. In the case-control study mentioned above, estimates of increased risk for patients treated with NOACs and these drugs varied and therefore it would be relevant to characterize the differences further in the data from study IV.

There was an increased risk of any severe bleed for patients treated with apixaban who were exposed to inhibitors of CYP3A4 and/or P-gp compared to those not exposed to the interacting drug group. However, the lower bound of the confidence interval approached one and therefore this result should to be interpreted with caution. No significant effect from exposure to inhibitors of CYP3A4 and/or P-gp could be found for patients treated with rivaroxaban or dabigatran regarding the primary endpoint, any severe bleed. For the secondary endpoint, gastrointestinal bleed, a significant effect was seen for patients treated with apixaban and rivaroxaban who were exposed to inhibitors of CYP3A4 and/or P-gp. Again, the lower bounds of the confidence intervals approached one. However, the summed result of analyses regarding inhibitors of CYP3A4 and/or P-gp indicate a potential effect on bleeding that could not be firmly established. There are two probable reasons why effect

measures could not be clearly established apart from the potential lack of effect. It is not unlikely that at least partly the relatively few patients available for analyses influence the results. Furthermore, it is possible that the distribution of interacting drugs within the groups of inhibitors of CYP3A4 and/or P-gp for each respective NOAC is unfavorably skewed towards drugs with less effect on NOAC pharmacokinetics. Furthermore, apixaban and rivaroxaban are substrates of CYP3A4 and P-gp. Dabigatran in contrast, is only a substrate of P-gp and not of CYP3A4 [32, 61]. This difference may have an impact on estimates of the risk of bleeding due to DDIs with inhibitors of CYP3A4 and/or P-gp. In the case-control study mentioned above, there were no statistically significant effects on major bleeding for NOAC-patients treated with inhibitors of CYP3A4 and/or P-gp [113]. However, increased incidence rates of major bleeding were seen for patients treated with NOACs and amiodarone, or fluconazole compared with NOAC alone in a large cohort study conducted on patients with atrial fibrillation in Taiwan [112]. But other findings of this study were unexpected. Treatment with NOACs and rifampin or phenytoin was associated with an increased risk of major bleeding. Both drugs are known to induce metabolism of NOACs. Furthermore, no association was seen for dronedarone, a strong P-gp and CYP3A4 inhibitor. Because of these results, the validity of this study have been questioned due to potential residual confounding [186-188].

Few patients were treated with NOAC and inducers of CYP3A4 and/or P-gp in the study population. The results of these analyses should therefore be interpreted very cautiously. For concomitant treatment with apixaban and inducers of CYP3A4 and/or P-gp there seemed to be an increased association regarding the secondary endpoint venous thrombosis, though the lower bound of the confidence interval was one. Since this was the largest group of patients analyzed for this endpoint it may signal that indeed, with a larger dataset or another methodology, more could be revealed. But results for inducers of CYP3A4 and/or P-gp regarding thromboembolism should primarily be seen as a starting point for further study and not be interpreted as either the potential presence or absence of an effect.

8.2 METHODOLOGICAL CONSIDERATIONS

8.2.1 Study populations

In all studies of this thesis the selection data sources and methods has been aimed at achieving large study populations. In study I and II the reason for this was directly connected to the research question and the aim of studying the whole Swedish outpatient population. The Prescribed Drug Register allows for a very close approximation of this since all pharmacies must report the data that constitute the register [118]. Though some patients may receive prescription drugs from other sources than pharmacies, e.g. directly from their healthcare provider, it is probable that this constitute a very small part of the drug use in the population and that patients not included in the study because of this are very few.

Furthermore, the aim of both studies was to describe frequently occurring potential DDIs and prevalence of DDIs, and it was assumed that patients not included in the study would not contribute significantly to differences in results would it have been possible to include them. Regarding external validity, the inclusion of approximately the whole outpatient population was a strength. In one sense instead of selecting a sample we studied the whole population. Whether results from Swedish outpatients can be applicable to the populations of other countries or for other time periods than the one studied is debatable. However, the objective of the studies was primarily to describe conditions in the Swedish outpatient population at the time.

In study III, the intention was to include patients more selectively. An important strength that was to be achieved was to increase the precision of the estimates regarding the interaction between warfarin and amiodarone compared to previous studies, and consequently a large population was needed. However, there was also a need to select patients that were on a documented stable warfarin treatment and who received amiodarone for a long enough period to avoid misclassification of exposed patients. Furthermore, to measure the effects information needed to be available from the data sources. Consequently, the widely used warfarin monitoring registers were selected as data sources as well as the Prescribed Drug Register, allowing a large population of patients to selectively evaluate for inclusion. Fairly elaborate inclusion and exclusion criteria were applied to ensure the above characteristics of the study population. The benefits of this were reduced risk of misclassification. However, a selection bias regarding the whole patient population exposed to the DDI may have been introduced by the inclusion only of patients for whom treatment were well documented. Since the aim was to characterize the effect of the DDI rather than to describe the outcome in the population in general, this weighing of potential sources of bias seemed reasonable.

In study IV, a large study population was needed explicitly to achieve power. This is not an uncommon challenge in observational studies of outcomes of DDIs. First, patients who are exposed to a combination of drugs that potentially interact may be few due to the known potential effect of the combination. Second, only a fraction of the patients exposed to an interaction are expected to experience the outcome. Consequently, we expected to need a large dataset to achieve power in the study and therefore evaluated all patients who had been dispensed NOACs from the introduction of these drugs in Sweden in the Prescribed Drug Register. The Patient Register, from which outcome data were retrieved, contained data until the end of 2017 at the time of retrieval and this restricted the population to patients who had been dispensed any NOAC between 2008 and 2017. This very inclusive first step was not only beneficial from a power perspective but also from an external validity perspective since a large proportion of the patients treated with NOACs in Sweden was considered for inclusion. NOACs are prescribed for patients with atrial fibrillation, DVT or PE, and after knee or hip replacement. Patients with other indications for treatment than atrial fibrillation

were excluded from the study population to avoid bias from differential distribution of patients with different risk profiles. However, the Patient Register, used for inclusion and exclusion, contains data from inpatient healthcare but not primary healthcare [120]. The diagnosis of atrial fibrillation is frequently established in primary healthcare whereas patients in primary healthcare are sent to the emergency room for diagnosis of DVT or PE, and knee or hip replacements are conducted in hospitals. Consequently, diagnoses for the exclusion criteria were expected to be more complete in the Patient Register than the potential inclusion criterion for atrial fibrillation. Therefore, to avoid misclassification and selection bias, atrial fibrillation was not used as an inclusion criterion. This would have resulted in a considerably smaller study population and would possibly systematically exclude the healthier atrial fibrillation patients treated in primary healthcare. However, by not including based on atrial fibrillation diagnosis it is probable that some patients who did not have the diagnosis were included and misclassified. The potential effect of this decision on the results was considered smaller than the effect a systematic exclusion of healthier patients might have had.

8.2.2 Exposure

In all studies Janusmed interactions was used to identify clinically relevant potential DDIs [5]. In the background text of this thesis, the impact of the selection of interaction database on the results of observational studies is described. Consequently, the choice of Janusmed interactions has implications for the definition of exposure to potential DDIs in studies I and II. The database is widely used in healthcare in Sweden and is integrated in many electronic medical records. The results of studies I and II can be interpreted both as a description of exposure to potential DDIs among patients and as an evaluation of concomitant use by prescribers, conscious or not, of drugs that potentially interact. For the second purpose, the choice of an interaction database that is well integrated in Swedish healthcare is a strength and can be considered an approximation of the information on potential DDIs available to prescribers. However, concerning the identification of potential DDIs that really are clinically relevant, Janusmed interactions have strengths and weaknesses. In studies I, II, and IV only class C and D interactions were included. As previously mentioned, class B interactions are potential DDIs with a clinical outcome that is uncertain and/or may vary. The exclusion of this group from the definition of exposure to clinically relevant potential DDIs is therefore a restriction of the potentially relevant DDIs found in the studies. The class was excluded because of the variable relevance of these interactions. Furthermore, the distinction between class C and D interactions is based on whether measures can be taken to mitigate the effect of the DDI (class C) or not (class D). Class D interactions are defined as DDIs that is best avoided, and risk being seen as the clinically most severe type of interactions. These aspects of the database translate into a problem of comparing results with those based on some other databases, e.g. the prevalence of moderate and severe interactions based on Stockley's Drug Interactions [85]. Essentially, B, C, and D interactions may all potentially be severe. However, this problem is not unique to Janusmed interactions and the database has the

advantage of being developed to selectively include potential DDIs that are clinically relevant, a strength considering the definition of exposure in the studies of this thesis.

In study IV, Janusmed interactions and EHRA guidelines were used to define exposure to interacting drugs. All class C and D interactions relevant for NOACs were included in the definition of exposure to interacting drugs [5]. Furthermore, clinically relevant interactions with NOACs as defined by EHRA guidelines were included [27]. Interacting drugs were grouped based on the mechanism for interactions. The grouping was implemented to allow relevant analyses since according to power calculations there were very few single drugs that occurred frequently enough for single drug pairs to be analyzed individually. However, the grouping entailed heterogeneous groups of drugs with different expected effects on the endpoint. Consequentially, the grouping may reduce the probability of measuring effects that may exist for single drugs. This definition of exposure was therefore both a strength and a weakness in study IV.

Apart from Janusmed interactions, the definitions of exposure in all studies depended on the interpretation of data from the Prescribed Drug Register of dispensations of larger quantities of drugs at single time points that needed to be interpreted as time periods for treatment. A limitation of the use of the Prescribed Drug Register in pharmacoepidemiologic research is that specific dosing information is only available as text strings of label text. In large datasets the interpretation of these texts is very challenging to achieve. The DDD of each prescription is also available but may not correspond to the actual dose prescribed. In studies I, II and III, data on single dispensations were interpreted as four-month treatment periods. The rationale behind this assumption, as previously described, relied on the dispensation of long-term drug treatment every three to four months in Sweden. In studies I and II, any drugs dispensed during the four-month study period were considered concomitant treatment. By this definition, all drug dispensations that constitute ongoing drug treatment were assumed to be included. The drawback of this strategy was a risk of misclassification of concomitant treatment based on drug treatment periods that may be separated in time during the study period. In study III, to further establish ongoing treatment, a second and third dispensation were used as an inclusion criterion. This strengthened the assumption that patients were using the drug during the study period. In study IV, a more elaborate definition of exposure was developed. Previous examples can be found of using the DDD of the dispensation to define the time period of treatment [147]. This practice was combined with the assumption of at most three to four months of intended use for each prescription to avoid overestimation of the treatment period. Consequently, the DDD of each dispensation defined the treatment period of each dispensation. A new dispensation that occurred within 2.5 x the DDD of the last treatment period was assumed to be connected unless it occurred more than 100 days after the last dispensation date. A strength of this method was that the risk of falsely connecting smaller dispensations was reduced. Furthermore, by multiplying with 2.5 the risk of not

connecting dispensations of drugs where half the DDD had been prescribed for a longer time-period was also reduced. However, a risk of misclassification of ongoing treatment remains as long as the true dosing information is not available.

Another important aspect of exposure, relevant to all studies in this thesis, is the assumption that dispensed prescription drugs correspond to consumed prescription drugs. It is well known that this is not always the case [189]. However, it is not realistically feasible to establish adherence to treatment in large study populations based on retrospective prescription or dispensation data and this is an inherent limitation in all large studies conducted in this type of data. The consequence is misclassification of patients that are assumed to use the drugs dispensed but do not take them. In studies I and II, the results are not only interesting from the perspective of what the patient actually consumed but also from the perspective of the intended treatment. What is measured is in fact the intended treatment from healthcare providers and the patient combined at the point of dispensation. In studies III and IV, the difference between dispensed drugs and consumed drugs is more purely a problem of misclassification. In study III, repeated dispensation of amiodarone is used as an inclusion criterion and repeated INR measurements confirm the use of warfarin. However, to some extent nonadherence to amiodarone may reduce the measured effect in the study and this is a limitation in the design that would be difficult to eliminate. In study IV, a similar problem of potential misclassification of exposure occurs and potentially result in reduced measures of effect size. However, in this specific aspect the population studied can be compared to an intention-to-treat population in a clinical trial. What is essentially measured is the effect for patients that are prescribed and dispensed the potentially interacting drugs concomitantly, not for the patients that actually consumed them, i.e. the effect in the population of combining these drugs as a treatment.

8.2.3 Covariables

In studies I and II the covariables included were fairly limited. Age, sex, and the number of prescribed drugs were included and are often included in similar studies [147, 160, 190]. Comorbidities were not included as covariables which is a limitation of studies I and II. This would have been relevant information to include in the analysis and previous research have shown that the number of chronic conditions is associated with the risk of being exposed to potential DDIs [160]. Furthermore, the age variable could be a proxy of the number of drugs used or comorbidities. However, only data from the Prescribed Drug Register were used and it does not include information on comorbidities.

In study III and IV, dosing information on amiodarone and NOACs was not available due to the limitation mentioned above in the Prescribed Drug Register. Amiodarone dose is

inversely correlated with warfarin dose according to previous research [105, 183]. Furthermore, dose adjustments of NOACs to mitigate the effect of potential DDIs is obviously a relevant factor in the analysis of risk of adverse events [27]. The absence of dosing information is therefore a limitation in both study I and IV. However, in study III, since the initiation of amiodarone is very standardized in Sweden it was assumed that the results reflect this dosing regimen. In study IV, the lack of dosing information as a covariable has consequences for the conclusions that can be drawn. It is reasonable to assume that the effect of potential DDIs are mitigated to some extent by dose reductions. Including the NOAC dose as a covariable would therefore be highly relevant. The results are, however, estimates of the risk connected with concomitant treatment, including potential dose adjustments, in the population and therefore describe the risk from an overall treatment perspective.

In study IV, covariables for comorbidities were based on CHA₂DS₂-VASc and HAS-BLED criteria. The components of these scores are well established as relevant for risk of thromboembolism and bleeding respectively and are used as covariables in similar studies [113, 133]. Other factors that may be relevant exist, e.g. some forms of cancer or smoking status [113]. However, limiting the choice of covariables for comorbidities to the components of the two scores was considered a reasonable balance of including established relevant covariables and avoiding the risk of overfitting the models. INR was not included as a covariable as it was not available in the utilized registers. Furthermore, some of the risk related to comorbidities, especially regarding HAS-BLED, can be mitigated by treatment in the clinic. This aspect could not be traced in the data and included in the definition of covariables.

8.2.4 Outcomes

Studies I and II were descriptive to a large part and frequencies of clinically relevant potential DDIs in the population were the focus of these studies. The prevalence of potential DDIs, related to the whole population or to patients prescribed at least two drugs is often reported in similar studies [10, 145, 147, 191]. The comparability of prevalence measures based on different interaction databases can be questioned as discussed above, but prevalence as a summary of occurrence in the population is well established and useful in comparisons between studies. Therefore, it is a limitation in paper I that no prevalence was calculated. This was done for the results section of this thesis and for the publication of paper II. Furthermore, a relevant outcome that was not included in paper I but calculated for paper II is the number of potential DDIs per patient. However, due to the different nature of different interaction databases and to achieve a detailed description of frequently occurring potential DDIs it is a strength of both study I and II that the outcome of focus was frequencies of potential DDIs in the population. In addition, as previously mentioned there are more than one way of

interpreting the results. One way of seeing it is that what is described is the burden of potential DDIs in the patient population. Another way of seeing it is that what is described is the extent to which prescribers combine potentially interacting drugs, knowing or not that they may have clinical relevance. For the purpose of the second way of seeing the outcomes, studies I and II provide inventories that are valuable for further discussion and analysis.

In study III, the primary outcome was change in warfarin dose after initiation of amiodarone compared to before. In addition, the change in INR during the study period was described. The purpose of the study was not to describe the potential increase in risk that may result from the interaction. Rather, the implications for dosing and monitoring of warfarin were the focus. However, a relevant outcome to consider would have been the risk of bleeding, comparing risk before and after initiation of amiodarone. Inclusion of this hard endpoint would probably result in a power problem with the size of the population studied and the design of the study might not be the ideal to study such an endpoint. Rather a design similar to that of study IV may be appropriate. Consequently, this endpoint was not included in study III.

Two composite endpoints were chosen as primary outcomes of study IV and selected components of these endpoints with the addition of venous thrombosis were secondary outcomes. These outcomes were all defined based on relevant ICD-diagnoses that were identified in the Patient Register and Cause of Death Register. The Patient Register, which was the source where most of the outcome data could be found, does not include diagnoses from primary healthcare. Furthermore, outcome diagnoses were only included and searched for that would correspond to severe cases. E.g. the primary endpoint “any severe bleed” did not include less severe bleedings. By these definitions and the use of the Patient Register for identification of outcomes we restricted all endpoints to severe cases. This was both a practical and intentional decision. First, since less severe diagnoses would not fully be found in the Patient Register for the study population and no corresponding data source exists for primary healthcare. Second, because severe bleeding or thromboembolic events were considered to be of primary relevance for the analyses. Therefore, the limited availability of events that did not require hospital care was not considered a limitation to the design.

8.2.5 Cross-sectional design in study I and II

The aim of studies I and II was to describe a cross-section of each study population regarding potential DDIs. The method used to identify concomitant drug treatment was to include all dispensed prescribed drugs from the Prescribed Drug Register during a four-month period. Whether the four-month period used yields a cross-section and whether prevalence calculated is a point prevalence can be discussed. The rationale behind the selection of the four-month

period has been described above. In short, exposure to concomitant drug treatment was defined based on the assumption that ongoing treatment is renewed every three to four months. By eliminating duplicate prescriptions for individual patients, the frequencies calculated for potential DDIs are therefore approximates of treatment at one time-point. Potential misclassification aside, this corresponds to a cross section and prevalence calculated could therefore be defined as a point prevalence. However, drug treatment that occur during shorter time periods or changes in treatment regimens during the four-month period may result in misclassification, both in relation to the point prevalence and regarding the exposure to potential DDIs. From that perspective the prevalence calculated could rather be regarded as a period prevalence, though because of the short timespan a measure of period prevalence very close to the point prevalence. The assumption that a four-month period corresponds roughly to a cross section in Swedish outpatient data from the Prescribed Drug Register is not unique to studies I and II and a validating study of this assumption would be highly relevant for the use of this method. Methods that confirm ongoing treatment by repeated dispensations or estimate exposure by taking the DDD into account, of which studies III and IV are examples, are probably more precise.

8.2.6 Longitudinal follow-up and statistical analyses in study III

In the primary analysis of study III, the difference in warfarin dose between a four-week period before amiodarone initiation and a four-week period during amiodarone treatment was analyzed. The period during amiodarone treatment was selected based on the descriptive analysis of weekly changes in dose during concomitant treatment. A period at which the dose change seemed to have levelled out was selected. This way of defining the comparison period for the primary analysis was pre-specified and therefore it may be argued that the analysis was not conducted post-hoc, though it was decided based on the longitudinal analysis. However, should the comparison time-point have been pre-established, it could be regarded as more stringent, but may have resulted in a false measure of the change in dose. Regardless, the aim of the study was to characterize the longitudinal dynamics of warfarin dose and INR after initiation of amiodarone and consequently the focus of the study was primarily descriptive.

For the purpose of comparing the change in dose between patients with a high and low warfarin dose at baseline, the primary analysis was repeated for each of these groups. The result was estimates of the mean percentage of change in dose for each group. Notably, the groups were not compared in the analysis. Such an analysis could have been informative and would have provided a statistical measure of whether there was a difference between the groups or not. However, by analyzing the mean change for each group the percentages of change could be calculated and compared. For descriptive purposes this was a clear way of analyzing the change in each group, and the difference was small with overlapping

confidence intervals. Though, if the difference had been larger, a statistical test might have added to the understanding of whether this difference was statistically significant.

8.2.7 Survival analyses in study IV

For analyses of the association of exposure to potential DDIs with bleeding or thromboembolic events in patients treated with NOACs, survival analyses with Cox-regression was conducted. Similar studies have used Cox-regression but also other methods e.g. logistic regression or Poisson regression [112, 113, 133]. Furthermore the propensity score has been used for stratification or inverse probability of treatment weighting [112, 133]. In study IV, survival analysis was considered appropriate to account for the potential difference of time to event between the comparison groups. In addition, because not all patients could be expected to be exposed during the whole study period, it was important to use a method that allowed for censoring. Since the Cox-regression model does not presume any assumptions about the shape of the hazard function or the distribution of survival times, it was selected as the more appropriate method for analysis. Propensity score stratification was considered while designing the study. The propensity score in study IV would be the probability that a patient received interacting drugs conditional on baseline characteristics [192]. It is not unlikely that differences in baseline comorbidities may lead to different propensity to be exposed to potential DDIs in the study. However, only observable covariables can be included in the propensity score. With a very heterogenous dataset due to multiple different treatments it is unlikely that the propensity score would capture the real propensity and hidden bias may still be present. Furthermore, computing the propensity score may result in increased variance in the estimate of the final analysis. Additionally, covariables included in the propensity score would not be included in the Cox-regression analyses and therefore the effects of these covariates would not be available for evaluation. Because of these aspects, any analyses including the propensity score would have been best suited as sensitivity analyses in study IV. Though it was decided for pragmatic reasons not to proceed with sensitivity analyses based on the propensity score, this could have been an interesting way of exploring the results further.

In study IV, 27 analyses of separate but overlapping datasets were conducted. For each analysis the first treatment period with the relevant NOAC for each patient was selected. If, at the start of the period, the patient was exposed to the interacting drug group studied in the analysis the patient was included in the group exposed to the potential DDI. Consequently, patients could contribute with different treatment periods in different analyses if they received more than one NOAC during the full study period and patients could be included in the group exposed to the potential DDI in one analysis and in the control group in another. Because of the large number of tests conducted, adjustment for multiple testing was considered. Whether adjustment for multiple testing is appropriate in observational studies have been discussed

[193, 194]. In short, if multiple tests are performed, the risk of false rejection of the null hypotheses increases due to chance. However, it can be argued that there is a fundamental difference between multiple comparisons conducted in the same dataset, combined to prove an overarching hypothesis, and comparisons conducted in different datasets to prove separate hypotheses [193]. Furthermore, the reduction of the risk of type I error achieved by adjustment for multiple testing increases the risk of type II error. For observational studies, it has been argued that this is not an insignificant problem and that routine application of multiple testing techniques ignore the purpose of observational research which is to find and describe associated phenomena in nature [194]. Considering these aspects, it was decided not to perform adjustments for multiple testing. Indeed, in study IV this would have had a pronounced effect of increasing the risk of type II error. Consequently, the results should be regarded as exploratory.

In study IV, all patients treated with NOACs available in the Prescribed Drug Register from the introduction of NOACs in Sweden and until 2017, the last year for which outcome data were available in the Prescribed Drug Register, was considered for inclusion. Consequently, to achieve a considerably larger dataset, patients from other countries would have had to be included. However, based on power analyses it was concluded that this large dataset was not sufficiently large to study combinations of single drugs, which would have been preferred. Furthermore, power analyses indicated that significant results for any difference between groups present for inducers of CYP3A4 and/or P-gp would not be achieved. Based on the power analyses, the grouping of potentially interacting drugs based on mechanisms for interactions was selected to achieve the objectives of the study. The grouping of different drugs introduces the problem of differential effects within the group. This is an important limitation of the study as the effects measured are not the effects of any specific DDI but rather the combined effects in relation to the proportion of contributing interacting drugs within each group. Though the effects of all potentially relevant interactions would not be possible to evaluate based on the available data, results would still have the potential to contribute to understanding of the risks of potential DDIs for atrial fibrillation patients treated with NOACs. As more patients accumulate over time in sources for retrospective analyses, similar studies can be conducted with higher precision. However, based on the available data the results of study IV contribute with an important estimation of the impact of DDIs in this patient group.

9 CLINICAL IMPLICATIONS AND CONCLUSIONS

A limited number of drugs were involved in a large fraction of the clinically relevant potential DDIs found in the whole population and the pediatric population. It is consequentially important that prescribers are aware of these drugs and evaluate potential DDIs to avoid adverse effects or reduced treatment effect. Furthermore, since the potential clinical impact of these drugs is large in the population it needs evaluation regarding actual clinical consequences for patients.

One fifth outpatients prescribed at least two drugs were exposed to clinically relevant potential DDIs. For the pediatric population of outpatients, the prevalence was considerably smaller. Comparisons to previous Swedish studies or international research are not easily made due to large differences in methodology. However, the potential clinical impact is large, this necessitates awareness among prescribers of interacting drugs and evaluation regarding clinical impact.

The most frequent potential effects of DDIs were bleeding, cardiac arrhythmias, and serotonin toxicity, and reduced effects of analgesics, anti-infectives, anticoagulants, anti-hypertensives, drugs for osteoporosis, and thyroid hormone substitution. For pediatric outpatients in addition to much of the above, toxicity related to opioids, contraceptives, anticonvulsants, or cytostatic drugs, and reduced effects of anticonvulsants and benzodiazepines were also frequent. These adverse events constitute a list of clinical effects for which DDIs should be considered among differential diagnostic options, especially in patients with polypharmacy.

The initiation of amiodarone in patients treated with warfarin may result in supratherapeutic anticoagulant effect with a peak within three weeks. It is therefore important to monitor frequently during initiation of concomitant treatment. Furthermore, a dose reduction of 25 % on average can be anticipated, but a large interindividual variation calls for caution. Monitoring within the first weeks of concomitant treatment was relatively high, but a fraction of patients did not have INR measured within the first weeks and increased awareness of the interaction may be needed. Continued frequent monitoring may be needed as the full effect of the interaction on warfarin dose continue to develop over many weeks.

The risk of bleeding events was increased for patients with atrial fibrillation treated with NOACs and drugs that interact pharmacodynamically or by inhibition of CYP3A4 and/or P-gp. This was only shown for apixaban regarding inhibitors of CYP3A4 and/or P-gp but it is not unlikely that there may be similarities between NOACs. However, pharmacokinetic

differences exist and further investigation in larger datasets is needed. The effect of inducers of CYP3A4 and/or P-gp could not be established, most probably due to small patient numbers, and therefore needs further investigation. From a clinical perspective, the literature on clinical impact of DDIs on bleeding and thromboembolism is limited and the results reported here expand it to some extent. Caution regarding all the studied groups of interacting drugs is called for. Though situations exist when the benefits of some of the combinations of drugs leading to these interactions outweigh the risks, they should be avoided if options are available.

10 REFLECTIONS ON FURTHER RESEARCH

10.1 GENERAL REFLECTIONS ON FURTHER RESEARCH

As described in the section on clinical relevance of DDIs above, research on the clinical consequences of DDIs is difficult to conduct on a general level. To a large extent, designing studies that correctly defines outcomes of a large variety of potential clinical effects and finding ways to gather this information efficiently is difficult. Previously used outcomes, e.g. hospitalization or documentation of a DDI connected to adverse events, are rough estimates that are open to misclassification. Therefore, specific studies on the clinical impact of potential DDIs where evidence is lacking is important. However, general estimates of clinical impact of DDIs may also be valuable if relevant designs can be identified. This type of studies may serve the purpose of educating prescribers of the relevance of DDIs and to highlight therapeutic areas where DDIs lead to morbidity and may be avoided. This is therefore an interesting and challenging field of study that needs further attention, especially concerning outpatient populations in which studies are rare.

The quality of evidence behind potential DDIs in Janusmed interactions and other databases vary greatly. Results of studies I and II, and similar studies in the literature, highlight interacting drugs frequently used concomitantly. With frequent use of potentially interacting drugs, the question regarding correct assumption of the clinical relevance of these interactions can be raised. Consequently, frequently occurring potential DDIs where evidence of the assumed clinical effect is weak are relevant for follow up in observational studies regarding their association with the potential outcome, and this is an important area for further research.

Studies on the clinical impact of DDIs in general, in patients treated with NOACs, are few in the literature. Much of the information on DDIs in guidelines and recommendations rely on pharmacokinetic studies, which is an important and fundamental source of information on DDIs. However, as more patients are treated with NOACs, data available for retrospective observational studies accumulate and will allow more detailed studies on both the general clinical impact of DDIs, and on clinical impact of concomitant treatment with less frequent specific drugs potentially interacting with NOACs.

10.2 FUTURE STUDIES

Studies I and II were conducted based on a cross-section of the Swedish population from 2010. Since then, many new drugs have been introduced to the market, recommended treatment options have changed, and the evidence regarding clinically relevant DDIs have expanded. A new study with similar aims is currently discussed. The study would give an updated description of exposure to potential DDIs in the population. Important aspects to

revisit and consider in the design of this study will be the definition of exposure to concomitant treatment, how to define a relevant cross section, and whether a relevant measure of clinical consequences of interactions can be developed to assess clinical impact of DDIs.

Frequencies of drugs were highly variable in the interacting group with pharmacodynamic effect in study IV. ASA was very frequent, clopidogrel and some of the NSAIDs were quite frequent, whereas prasugrel and ticagrelor were less frequent. A study of the potential difference in risk for patients with NOAC treatment exposed to these individual drugs based on the dataset of study IV has been discussed and will be evaluated regarding feasibility and appropriate design. The identification of a relevant methodology to achieve power will be an important issue to consider in the design of this study.

In study IV, the potential association between exposure to inducers of CYP3A4 and/or P-gp and thromboembolic events could not be estimated due to lack of power. A potential way of studying this, based on the dataset from study IV, may be by a case-control study. This option has not been evaluated from a feasibility perspective but may potentially be a way forward to address this research question.

A slightly different approach to exposure to potential DDIs, based on the dataset from study IV, could be to focus on the potential association between comorbidities and exposure to interacting DDIs. It is not unexpected that previous research has indicated that comorbidities increase the risk of being exposed to a potential DDI. A characterization of the types of potential DDIs that may be associated with different comorbidities could be relevant. The purpose of studying this would be to increase awareness of frequent DDIs in different subsets of patients treated with NOACs. However, this idea has not been further outlined and require thorough evaluation regarding relevant research questions, design, and feasibility.

11 POPULÄRVETENSKAPLIG SAMMANFATTNING

Inledning

Behandling med läkemedel är ett centralt verktyg i sjukvården, men fördelarna med behandlingen måste alltid vägas mot riskerna. För patienter som behandlas med mer än ett läkemedel förekommer det ibland att de olika läkemedlen påverkar varandra, så kallade läkemedelsinteraktioner. De kan vara önskvärda, interaktionen bidrar då till en ökad effekt mot det man vill behandla. Vanligare är dock att läkemedelsinteraktioner är oönskade, konsekvenserna kan då vara ökad risk för biverkningar eller minskad behandlingseffekt.

I denna avhandling undersöks i vilken utsträckning läkemedel som interagerar med varandra, och där interaktionen kan leda till betydande effekter, kombineras i svensk öppenvård, det vill säga bland patienter som inte är inlagda på sjukhus. Även effekter av interagerande läkemedel vid blodförtunnande behandling undersöks.

Bakgrund

Läkemedelsinteraktioner utgör ett komplext problem. Vissa kombinationer av läkemedel kan interagera med varandra utan att effekten är så stor att det har någon betydelse för behandlingen. Andra kombinationer kan ha så betydande interaktioner att biverkningar, toxicitet eller förlust av behandlingseffekt innebär livsfarliga konsekvenser för patienten. En ytterligare komplicerande aspekt är att inte alla patienter får en effekt som har betydelse även om de två läkemedel som används har en mekanism som kan ge en betydande interaktion. Man talar därför om potentiella läkemedelsinteraktioner, det vill säga interaktioner som innebär en risk för en oönskad effekt. Ibland är det dessutom så att man väljer att kombinera två läkemedel som man vet interagerar för att man inte har några behandlingsalternativ för patienten. I vissa fall, men inte alltid, kan man då kompensera för interaktionseffekten genom att till exempel justera dosen av det läkemedel som påverkas. Man talar om kliniskt betydande potentiella interaktioner när det är sannolikt att effekten av interaktionen har betydelse för patienten.

Det finns två principiellt olika typer av läkemedelsinteraktioner. Farmakokinetiska interaktioner uppkommer genom att ett läkemedel påverkar omsättningen av ett annat läkemedel i kroppen. Kroppen gör sig då av med det andra läkemedlet fortare eller långsammare, vilket leder till större eller mindre mängder av det läkemedlet i kroppen. Det i sin tur har betydelse för behandlingseffekt och biverkningar. Farmakodynamiska interaktioner är istället sådana där de interagerande läkemedlens effekter eller biverkningar samverkar eller motverkar varandra. Man kan bland båda dessa typer av interaktioner också skilja på sådana som ger en risk för biverkningar respektive en förlust av behandlingseffekt.

Avhandlingen har en särskild inriktning på effekt av interaktioner vid behandling med orala antikoagulantia, dvs blodförtunnande läkemedel. De blodförtunnande läkemedel som studeras är warfarin, som funnits länge, och de läkemedel som ingår i den nyare läkemedelsgruppen NOAK. Dessa läkemedel är apixaban, rivaroxaban, dabigatran och edoxaban. I en av studierna undersöktes effekterna av interaktionen mellan amiodaron, ett rytmreglerande läkemedel, och warfarin. Amiodaron påverkar omsättningen av warfarin och leder till ökad mängd av läkemedlet i kroppen, man kan därför behöva sänka warfarindosen vid samtidig behandling för att undvika blödning. Effekten av läkemedelsinteraktioner med NOAK undersöktes i en studie där både risken för blödning och trombos, dvs blodpropp analyserades. Interaktioner med andra läkemedel som precis som NOAK har blodförtunnande effekt kan leda till ökad blödningsrisk. Interaktioner med läkemedel som ökar mängden NOAK i blodet kan också leda till ökad blödningsrisk. Slutligen kan interaktioner som minskar mängden NOAK i blodet ge minskad behandlingseffekt av NOAK och därmed risk för trombos.

Syfte

Avhandlingens syfte var att öka kunskapen om förekomst i svensk öppenvård av samtidig behandling med läkemedel som man vet interagerar. Den syftade också till att undersöka hur insättning av amiodaron påverkar dosering samt den blodförtunnande effekten av warfarin. Dessutom syftade den till att undersöka hur risken för blödning och trombos påverkas av interagerande läkemedel hos patienter som får NOAK.

Metoder

Alla studier i avhandlingen utfördes baserat på data från olika register. Förekomst av interagerande läkemedelskombinationer undersöktes i två studier där all läkemedelsbehandling som förekom vid en specifik tidpunkt i början av 2010 studerades, så kallade tvärsnittsstudier. Den första studien genomfördes baserat på alla receptbelagda läkemedel utlämnade från svenska apotek till patienter i alla åldrar. Den andra studien genomfördes på en undergrupp av den första, baserat på patienter som var 17 år eller yngre. I båda grupperna studerades förekomsten av potentiella läkemedelsinteraktioner. Läkemedel som ofta förekom i kliniskt betydande potentiella interaktioner identifierades. Den tredje studien utfördes baserat på patienter som behandlas med warfarin och som inledde behandling med amiodaron. Detta var en kohortstudie vilket innebär att en grupp följs upp avseende det man vill mäta. Doseringen av warfarin efter insättning av amiodaron följdes veckovis under studieperioden. Skillnaden i dos jämfördes innan och efter dosjustering. INR, som är ett mått på den blodförtunnande behandlingseffekten av warfarin, följdes veckovis efter insättning av amiodaron. I den fjärde studien ingick alla patienter med förmaksflimmer

som hämtat ut något NOAK på apotek mellan 2008 och 2017. Även detta var en kohortstudie. Skillnaden i risk för blödning respektive trombos hos patienter som då de började använda NOAK också använde interagerande läkemedel jämfördes med de som inte använde sådana läkemedel. För interagerande kombinationer av läkemedel som förväntas leda till fler blödningar undersöktes om risken för blödning ökade. För de kombinationer som förväntas leda till försämrade effekt av NOAK studerades om risken för trombos ökade.

Resultat

Bland patienter med minst två läkemedel i hela den svenska öppenvårdspopulationen hade 19 % minst en kliniskt betydande potentiell läkemedelsinteraktion. Bland barn upp till 17 år gamla med minst två läkemedel hade 1,4 % en kliniskt betydande potentiell läkemedelsinteraktion. Ungefär hälften av kliniskt betydande läkemedelsinteraktioner var av den typ som potentiellt kan leda till förlust av behandlingseffekt i studien av alla åldrar medan andelen var lägre bland barn. Ett begränsat antal läkemedel var involverade i en stor andel av alla kliniskt betydande potentiella läkemedelsinteraktioner.

Den genomsnittliga veckodosen av warfarin var 24,6 % lägre under behandling med amiodaron, jämfört med innan insättning av amiodaron. Det fanns inga skillnader avseende denna dossänkning relaterat till ålder eller kön. Genomsnittligt INR per vecka var som högst den tredje veckan efter att amiodaronbehandling hade inletts. 37,1 % av patienterna hade då ett INR som låg över det intervall man strävar efter och deras risk för blödning var därmed större än önskvärt. Innan amiodaron sattes in var denna andel 11,7 %.

Patienter som behandlades med läkemedel som har farmakodynamiska interaktioner med NOAK hade en ökad risk för blödning jämfört med de som inte fick sådana läkemedel. För läkemedel som kan öka mängden NOAK i blodet var risken för blödning ökad hos patienter som tog apixaban. Det var mycket få patienter som använde NOAK tillsammans med läkemedel som minskar mängden NOAK i blodet och effekten av dessa kombinationer kunde inte fastställas.

Slutsatser

Ett begränsat antal läkemedel är involverade i en stor andel av kliniskt betydande potentiella läkemedelsinteraktioner. Uppmärksamhet på dessa läkemedel och hur de används i kombination med andra läkemedel kan därför bidra till patientsäkerheten. En stor andel av svenska öppenvårdspatienter behandlas med läkemedelskombinationer som kan leda till kliniskt betydande interaktioner. En stor andel av dessa interaktioner kan leda till förlust av

behandlingseffekt. Det är tänkbart att förlust av behandlingseffekt är ett underskattat problem relaterat till läkemedelsinteraktioner och det är därför viktigt att vara uppmärksam på denna effekt. Det finns ett stort potentiellt värde i att minska onödig samtidig användning av interagerande läkemedel och att noggrant värdera risker och nytta med sådan behandling.

Då behandling med amiodaron inleds hos patienter som behandlas med warfarin krävs ofta en reducerad dos för att undvika överbehandling med warfarin. Frekvent uppföljning med INR-mätningar och justering av warfarindosen är viktigt för säker behandling. Den förväntade genomsnittliga dosreduktionen kan förväntas vara ca 25 %. Det är dock viktigt att vara uppmärksam på att skillnaden är stor avseende hur mycket dosen behöver förändras mellan olika patienter.

Behandling med läkemedel som interagerar genom att samverka till en blodförtunnande effekt, eller med läkemedel som interagerar genom att öka mängden NOAK i blodet leder till ökad risk för blödning hos patienter som behandlas med NOAK vid förmaksflimmer. För läkemedel som ökar mängden NOAK i blodet kunde detta bara visas i kombination med apixaban men det är inte osannolikt att det finns likheter för andra NOAK. Detta behöver undersökas närmre i ytterligare studier med fler patienter. För läkemedel som minskar mängden NOAK i blodet kunde inte någon effekt fastställas och även detta behöver undersökas vidare.

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